and contains several ORs, OBPs and IRs. These genes are candidates for being involved in the adaptation of *An. gambiae* to its human host and were sequenced in six anopheline species to identify those that show evidence of positive selection.

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DENGUE 2 INFECTION ALTERS MICRORNA EXPRESSION IN *AEDES AEGYPTI*

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Emerging studies show that important avenues in the post-transcriptional regulation of gene expression occur via small RNA regulatory pathways. Non-coding RNAs (ncRNAs) are key features of these pathways, and critical molecules involved in their biogenesis and function are conserved in plants, insects and mammals. To identify products of anti-viral RNA interference in vector mosquitoes, deep sequencing small RNA (sRNA) libraries were prepared from DENV2-fed *Ae. aegypti* females (RexD strain) and matched un-infected controls at 2, 4, and 9 days post-infection (dpi). An earlier publication described DENV2-derived viral sRNAs (viRNAs) across three size classes: unusually small RNAs (usRNAs) (14-19nts), canonical siRNAs (20-24nts), and piRNAs (25-30nts) (Hess et al, BMC Microbiology, 2011). In the present analysis, these libraries were mined to determine whether substantive changes occur in mosquito microRNA (miRNA) levels during DENV2 infection. Reads were aligned to mIRBase release 17 hairpin database (mirbase.org). MiRNAs with over 50 reads across treatment groups and showing $\geq$ 2 fold-changes were chosen for further study. Our analysis reveals that significant changes to specific miRNA levels occur in DENV2-infected mosquitoes compared to un-infected controls. Moreover, some miRNAs showed coordinated enrichment or depletion at both 2 and 4 dpi, substantiating the hypothesis that they are important to the establishment of virus infection, whether by being exploited by the virus or as part of an anti-viral mechanism. Coordinately co-regulated miRNAs or *miRNAs include miR155, mIR2755, miR281 and miR277, among others. miRNAs were classified by type: conserved (homologous to previously reported miRNAs), *miRNA (complementary to miRNA), non-canonical or unclassified. Although the precise part played by each differentially expressed miRNA remains to be elucidated, orthologous miRNAs in other animals are important effectors of cellular differentiation, neurogenesis, transcriptional regulation, nutritional metabolism and the regulation of apoptosis. Our results show an intriguing new way in which major cellular processes of mosquitoes respond to arbovirus infection.

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GENETIC REGULATION OF VECTOR MOSQUITO SALIVARY GLAND DEVELOPMENT

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Understanding mosquito salivary gland development is critical given the importance of this tissue in blood feeding and pathogen transmission. Our recent survey of the mosquito genomes indicated that mosquitoes have orthologs of many genes that regulate embryonic salivary gland development in *Drosophila melanogaster,* a well-characterized insect genetic model organism. The expression patterns of a large subset of these genes were assessed during development of *Aedes aegypti,* an emerging model for vector mosquito development. These studies revealed that the early stages of *Ae. aegypti* salivary gland development significantly differ from that of *D. melanogaster.* We are now using an RNAi knockdown strategy to investigate the roles of genes expressed in the developing *Ae. aegypti* salivary gland. Functional characterization of *cyclic-AMP response element binding protein A (crebA)* indicates that this gene encodes a key regulator of secretory function in the *Ae. aegypti* salivary gland. These studies highlight the need for further analysis of mosquito developmental genetics and may foster comparative studies of salivary gland development in additional vector insect species.

1003

MEDUSA: A NOVEL GENE DRIVE SYSTEM FOR CONFINED SUPPRESSION OF MOSQUITO POPULATIONS

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Following successful field trials of sterile GM mosquitoes designed to control dengue fever, interest is now growing in the use of gene drive systems, such as X-shredders, capable of inducing a population crash as they spread. These systems hold much promise for wide-scale disease control; however issues arise from their potential to spread across international borders. We propose a novel gene drive system, Medusa, capable of inducing a local population crash without spreading into neighboring populations. Medusa consists of four components - two at a locus on the X chromosome and two at a locus on the Y chromosome. A maternally-expressed, X-linked toxin and a zygotically-expressed, Y-linked antidote suppress the female population because only males can protect themselves against the effects of the toxin. A zygotically-expressed, Y-linked toxin and a zygotically-expressed, X-linked antidote ensure that the two constructs are always inherited together. We use simple population genetic models to explore the dynamics of the Medusa system. An all-male release is preferred since males don't bite and, if released over two generations, Medusa is expected to induce a population crash within seven generations for modest release sizes. Re-invasion of wild mosquitoes can lead to the population eventually rebounding; however this can be prevented by small, regular releases of Medusa males. The Medusa system could serve as a proof of principle for invasive population suppression systems such as X-shredders. We describe molecular solutions to chromosomal anomalies that could interfere with Medusa dynamics.

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VACCINATION WITH EXCRETORY/SECRETORY PRODUCTS CONFERS PARTIAL PROTECTION IN A MURINE MODEL OF FILARIASIS

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Infection with filarial worms can cause severe and debilitating diseases in both humans and animals. While many vaccine candidates have been studied in filariasis, our understanding of protective immune responses in a permissive model to filariasis is incomplete. In this study, we evaluated preparations of worm antigens for protection against challenge infection in the BALB/c Litomosoides sigmodontis model. The fractions included LS, soluble antigens of a homogenate of adult worms, and ES, excretory/secretory products of adult female worms. 6-8 week old female BALB/c mice were given 3 intraperitoneal injections of 10 micrograms LS or ES with CpG/Alum and subsequently challenged with 40 infectious larvae subcutaneously. 8 weeks after infection mice were euthanized and parasite burdens were determined. Mice that were vaccinated with LS antigen showed no significant protection against challenge infection compared to control mice. Mice vaccinated with ES product, however, harbored 60% fewer adult worms than control mice. While mice vaccinated with LS and ES produced similar levels of IgG antibodies to both antigen preparations, analysis by western blot demonstrated that ES vaccinated mice recognized different ES proteins than LS-vaccinated mice. Currently, we are in the process of conducting mass spectroscopy to identify a ~160kda protein that was strongly preferentially recognized by ES-vaccinated mice. No substantially large differences were observed between the two vaccinated groups with regards to lymphocyte

proliferation or Th1 and Th2 cytokine production in response to ES or LS. However, IL-10 responses to parasite antigens were substantially decreased in ES vaccinated mice. Analysis of worm counts at early timepoints suggest that ES vaccination does not disrupt L3 migration through tissues. Work is underway testing whether ES vaccination blocks the ability of filarial worms to induce immune regulation.

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IMMUNO-PROPHYLACTIC EVALUATION OF RECOMBINANT CUTICULAR COLLAGEN (COL-4) AND ABUNDANT LARVAL TRANSCRIPT (ALT) USING MULTIVALENT STRATEGY IN EXPERIMENTAL FILARIASIS

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Human lymphatic filariasis is commonly known as elephantiasis and is a profoundly disfiguring disease caused by the parasitic nematodes such as *Wuchereria bancrofti*, *Brugia malayi* and *B. timori*. There are no vaccines to aid control programmes although the current estimate for people living in endemic areas are in the range of billions over 81 countries. Nematode parasites have a complex life cycle and hence vaccine development efforts may require incorporation of antigens representing various stages to improve efficacy. In this regard, we have evaluated the vaccine potential of cuticular collagen (COL-4) that forms a major component of the nematode cuticle. Further, we have also investigated the protective efficacy of COL-4 in combination with L3 stage specific antigen ALT-2. Accordingly, the col-4 gene was PCR amplified from infective stage (L3) stage cDNA library of *W bancrofti* and was cloned in prokaryotic expression vector. The 15kDa recombinant His-tagged protein was over expressed by salt inducible system and was purified by metal-affinity chromatography. The human immune response of the rWbCOL-4 was analyzed with various clinical sera and was found to show significant reactivity with endemic normal sera (putatively immune). The immunoprophylactic studies were carried out in *Meriones unguiculatus* (Jird) model by immunizing COL-4 either alone or in combination with ALT. The COL-4 immunized group gave 75 % protection and interestingly the combination groups (COL-4 +ALT) were found to be significantly higher in the range of 85%. Humoral and cellular immune responses suggest more of Th2 type response in parasite clearance. Hence, we report for the first time the role of COL-4 as a putative vaccine candidate in eliciting protective immune response in experimental filariasis.

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THE *ACANTHOCHEILONEMA VITEAE* PRODUCT ES-62 SUPPRESSES PATHOGENESIS IN COLLAGEN-INDUCED ARTHRITIS BY TARGETING OF THE IL-17-PRODUCING CELLULAR NETWORK AT MULTIPLE SITES

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ES-62 is an immunomodulatory, phosphorylcholine-containing glycoprotein secreted by the rodent filarial nematode *Acanthocheilonema viteae,* which has been found to be protective in the collagen-induced arthritis (CIA) mouse model of rheumatoid arthritis (RA). As IL-17 has been reported to play a pathological role in the development of RA, we investigated whether targeting of IL-17 may explain the protection afforded by ES-62 in this model. The CIA model employs DBA/1 mice, which progressively display arthritis following immunization with type-II collagen. The protective effects of ES-62 were assessed by measurement of cytokine levels, flow cytometric analysis of cell populations and *in situ* analysis of joint inflammation. ES-62 was found to down-regulate a number of different IL-17 responses in the CIA model. Firstly, it acts to inhibit priming and polarisation of IL-17 responses by targeting a complex IL-17-producing network, involving signalling between dendritic cells and/or $CD4^+$ T cells. Secondly, ES-62 directly targets Th17 cells by down-regulating expression of MyD88 to suppress responses mediated by IL-1 and TLR ligands. Further, ES-62 interferes with migration of T cells and this is reflected by direct suppression of CD44 up-regulation and, as evidenced by *in situ* analysis, dramatically reduced levels of IL-17-producing cells, including lymphocytes, infiltrating the joint. Finally, there is strong suppression of IL-17 production by cells resident in the joint, such as osteoclasts within the bone areas. Targeting of IL-17 responses by ES-62 presumably reflects the need of the parasite to dampen down host inflammatory responses that could be dangerous to parasite or even host. However, the multi-site manipulation of the initiation and effector phases of the IL-17 inflammatory network is notable and we believe further strengthens the argument for exploiting this potent helminth-derived immunomodulator, in the development of novel therapeutics for RA.

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LITOMOSOIDES SIGMODONTIS INFECTION INDUCES BASOPHIL SUPPRESSION IN RESPONSE TO ALLERGEN IN A MOUSE OVALBUMIN ALLERGY MODEL

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Helminth infections have been shown to protect against allergic disease, yet the mechanisms by which this occurs are not fully understood. We have begun to evaluate the effects chronic helminth infections have on the ability of effector cells of allergy to respond to allergen. Mice were sensitized to ovalbumin (OVA) and then infected with Litomosoides sigmodontis for 10 weeks. To assess basophil responsiveness, whole blood of sensitized mice was incubated with OVA and basophil activation was quantified by intracellular IL-4 expression. 20% of basophils from OVA-sensitized mice expressed IL-4 above basal levels, while basophils from OVA-sensitized mice that were chronically infected demonstrated no increase in IL-4 expression. Stimulation with anti-IgE antibody also showed basophil suppression in chronically infected mice, suggesting that decreased basophil responsiveness to OVA was due to a general suppression in IgE-mediated signaling. Infection was also associated with decreased proliferative responses of splenocytes to anti-CD3/anti-CD28 stimulation and decreased IL-4 and IL-5 production in response to OVA. These findings suggest helminth-mediated protection is due to both direct effects on allergy effector cells as well as inhibition of signals that drive allergic responses. Though we found no difference in IL-10 production in response to OVA, infected animals had increased IL-10 production in response to parasite antigen. Interestingly, the effect infection has on basophils may be rapid, as preliminary studies demonstrate that implantation of adult worms can suppress basophils in as little as 3 days. Ongoing experiments are investigating the effects infection has on mast cell function and clinical allergic disease.

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INTERIM SUCCESS IN THE DEVELOPMENT OF A *LOA LOA* AND MICROFILARIA-SPECIFIC ANTIGEN TEST

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Severe adverse events in patients with high levels of circulating microfilariae of *Loa loa* treated with either ivermectin or DEC have halted mass drug administration campaigns in areas of Africa endemic for these filariae. To identify individuals at risk for severe post-treatment sequelae, diagnostics that are *Loa*-specific and quantitative for microfilariae are urgently needed. To identify and quantify microfilariae-specific protein targets for a rapid immunoassay, we performed trypsin digestion followed

by mass spectrometry of *Loa loa* microfilarial excretory/secretory (E/S) product. Microfilariae from 4 patients with loiasis were collected following therapeutic apheresis, purified, and then incubated for 24-48 hours in serum free media. The E/S product was then concentrated and analyzed by nanobore reverse-phase liquid chromatography-tandem-MS (nanoRPLC-MS/MS) following trypsin digestion, after which the obtained spectra were searched against the recently completed putative proteome of *Loa loa* using SEQUEST. Of 15462 potential proteins in the proteome, 1274 (8.2%) E/S proteins of *Loa loa* microfilariae were identified. Twenty-five of the most highly abundant proteins are currently being explored as targets for a molecular-based diagnostic. Moreover, these E/S proteins were cross-referenced with a nanoRPLC-MS/MS analysis of proteins found specifically in the urine of patients with loiasis (and not in normal control urine). Four urinary proteins were identified that had no or little homology to human proteins; these are also being assessed for their diagnostic utility. Additionally, rabbits were injected with concentrated E/S and specific Ll-ES antisera raised. Purified IgG from this Ll E/S-specific antisera has been used to develop a sandwich ELISA that can detect Ll ES in human sera. Our data suggest that E/S products of Ll can be detected in human blood; studies examining the utility of such an antigen assay are currently underway in hopes of having a quantitative, microfilaria-specific circulating antigen test for use in blood and/or urine.

1009

DYNAMICS OF ANTIBODY RESPONSES BY ISOTYPES AND IGG SUBTYPES IN LABORATORY MODELS FOR ONCHOCERCIASIS

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Onchocerca volvulus, the causative agent of "river blindness," infects approximately 37 million people worldwide. Of these infections, 270,000 may result in blindness and 500,000 in vision impairment. The surveillance mechanisms for the detection of *O. volvulus* infections in humans is based on an IgG4 Elisa test using the recombinant antigen OV-16. This study examined the immune responses against OV-16 by IgM, IgG and the four IgG isotypes. Six chimpanzees were laboratory inoculated with 200-400 microfilria of *O. volvulus* microfilaria, and serum samples and skin snips were collected monthly for 4-5 years post inoculation. Negative sera samples were assayed and the cutoffs for positivity were determined by their mean plus 3 standard deviations. The Elisa values from IgG1, IgG2, IgG3, and whole IgM levels were not adequate indicators of infection, either due to low dynamic spectrum of OD values or responses that were detected later than IgG4 or whole IgG. The OD values for IgG4 were positive at 13.8 months post inoculation (median=15.5, range=7-18), approximately 1.2 months before the animals became microfilaria positive. Elisa values for whole IgG become positive at 11.4 months post-inoculation (median=13, range=4-15), which was 3.6 months before skin snips were detected positive. Our findings indicate that whole IgG reactivity may detect infections 2-months earlier when using the IgG4 OV-16 Elisa.

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PROTEOMIC PROFILING OF MICROFILARIA-EXPOSED HUMAN DENDRITIC CELLS IDENTIFIES HOST PATHWAYS ASSOCIATED WITH DENDRITIC CELL DYSFUNCTION

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Dysregulation of antigen presenting cells such as dendritic cells (DC) and macrophages (MΦ) is one of the many mechanisms proposed to mediate the profound filarial-specific T cell hyporesponsiveness seen in lymphatic filariasis. In fact, we have previously shown that live microfilariae (mf) of *Brugia malayi* induce apoptotic cell death in human monocyte-derive DC (mDC) and also modulate their ability to activate T effector cells. To characterize more completely heretofore unrecognized pathways and/or processes in mDCs influenced by mf exposure, the proteomes of mf-exposed and -unexposed mDCs were analysed by nanobore reverse-phase liquid chromatography-tandem-MS (nanoRPLC-MS/MS) following trypsin digestion. The obtained spectra were searched against *Homo sapiens, B. malayi* and *Wolbachia* databases using SEQUEST. A total of 1395 proteins were observed to be 2-fold regulated (spectral abundance) in mf-exposed mDCs that significantly correlated ($p<0.0001$) with transcriptional data analyzed independently using human microarray analysis. Interestingly, compared to mf-unexposed DC, mf upregulated (by ~4 fold) the cell surface and soluble forms of ICAM-1, processes mediated by IL-8, IL-17F and TNF-α– pathways. Ingenuity™ pathway analysis suggested that mf significantly downregulated ($p< 0.0001$) the mammalian target of rapamycin (mTOR), eukaryotic initiation factor (eIF) 2, eIF4 and p70S6K pathways as well as metabolic pathways involved in pyruvate metabolism and protein ubiquination. Interestingly, live mf secrete homologues of human FKBP1 (cyclophilin) a negative regulators of mTOR signaling. Because mTOR inhibitors (e.g. rapamycin) induce apoptosis in human DC and down-modulate the DC function required for T cell activation, functional studies on the mechanisms of mf-induced inhibition of mTOR are underway to provide the mechanistic link between the internalization of mf antigens and dysfunction seen in human DC induced by mf.

1011

TETRAVAX-DV, A LIVE ATTENUATED TETRAVALENT DENGUE VACCINE, IS SAFE, HIGHLY IMMUNOGENIC, AND INDUCES PROTECTION AGAINST A CHALLENGE DOSE OF VACCINE

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Dengue virus (DENV) has become the most important arbovirus worldwide with approximately 36 million cases of dengue fever and more than 2 million cases of severe dengue occurring annually. Several live attenuated tetravalent DENV candidate vaccines are being evaluated in clinical trials and the most advanced candidate has entered Phase 3 trial in numerous dengue-endemic countries. TetraVax-DV, a live attenuated dengue vaccine developed by the NIAID has been evaluated in several Phase I trials in the US and is poised to enter a Phase 2 trial in Brazil. In developing TetraVax-DV, we first evaluated the safety, replication kinetics, and immunogenicity of 8 different monovalent dengue vaccines in 15 separate trials to identify those candidates most suitable for inclusion in a tetravalent vaccine formulation. We then evaluated 5 different tetravalent admixtures to determine which combination would generate the most suitable safety

and immunogenicity profile. In that study, TetraVax-DV TV003 was very well tolerated by flavivirus-naïve adult subjects and elicited a trivalent or better antibody response in 90% of vaccinated subjects following a single subcutaneous dose. Because of these early promising results, we decided to further evaluate the safety and immunogenicity of TV003. In addition, we evaluated the ability of a single subcutaneous dose of TV003 to protect against challenge with a second dose of vaccine given 6 months after the first dose. 56 subjects were enrolled in this trial; 40 subjects received TV003 and 16 received placebo. Each of the components of TV003 was given at a dose of 1,000 PFU. Six months after receipt of the first dose of vaccine, subjects were challenged with a second dose of vaccine (or placebo). Subjects were followed in an identical manner after first and second doses. Viremia and safety labs were assessed on days 0, 3, 6, 8, 10, 12, 14, and 16 after each immunization. Specimens were collected for serological analysis on study days 0, 28, 56, 90, 150, and 180 post-immunization. Following the first immunization, the vaccine induced a tetravalent neutralizing antibody response in 71% of vaccinees and a trivalent or better response in 92% of vaccinees. Complete safety and immunogenicity data following the first vaccination will be discussed. The safety, absence of viremia, and immunologic response of vaccinees to the challenge dose of vaccine will also be discussed.

1012

IMMUNOGENICITY AND SAFETY OF A TETRAVALENT DENGUE VACCINE IN CHILDREN AND ADOLESCENTS IN LATIN AMERICA

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A tetravalent dengue vaccine (TDV) comprising recombinant, live, attenuated viruses, one per serotype (CYD-1-4), is being evaluated for protective efficacy in phase III. The objective of this study was to assess the safety and immunogenicity of TDV in endemic areas in Latin America. A randomized, controlled, observer-blind (first and second injections) and single blind (third injection) phase II clinical trial was conducted among healthy children aged 9 to 16 years in Colombia, Honduras, Mexico and Puerto Rico (ClinicalTrials.gov CT00993447). Participants received either 3 doses of TDV (n=401) or 2 doses of saline followed by a dose of Tdap (Adacel) vaccine (n=199) at Months 0, 6 and 12. Solicited injection site and systemic reactions were recorded daily for 7 and 14 days respectively after each injection. Plaque reduction neutralization test ($PRNT_{50}$) antibody titers against the TDV parental viruses were measured before and 28 days after each injection. The median age of participants was 12.6 and 52% were females. No vaccine-related serious adverse events were reported. Solicited injection site and systemic reactions after the first injection were reported by 31.6% and 57.9% of subjects in the TDV group and 27.6% and 54.3% of subjects in the Control group. A decrease in reactogenicity rates was observed after the second and third TDV doses. Most reactions were mild. Injection site pain and headache were most frequently reported. Seropositivity rates [antibody titers ≥10 (1/dil)] one month after the 3rd TDV vaccination, were: 94.2%, 98.9%, 100%, and 98.9% for serotypes 1-4, respectively. The corresponding geometric mean titers were 320, 486, 594, and 273. The percentage of seropositivity after 3 TDV doses was 98.6% for at least 3 serotypes, and 93.4% for all 4 serotypes. A three-dose TDV regimen had a satisfactory safety and reactogenicity profile and elicited neutralizing antibody responses against all four serotypes in children and adolescents in Latin America. These results are consistent with those from prior phase I and II clinical trials.

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IMPACT OF PRE-EXISTING IMMUNITY ON THE SAFETY AND IMMUNOGENICITY OF DENVAX, A TETRAVALENT DENGUE VACCINE

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An effective vaccine against dengue viruses (DENV) must be safe, immunogenic, and capable of eliciting a long-term protective immunity against all four serotypes. Pre-existing immunity to flaviviruses can significantly impact the safety and immunogenicity of a tetravalent dengue vaccine. Here the safety and immunogenicity of DENVax, a novel tetravalent dengue vaccine, was assessed in previously dengue-exposed AG129 mice (deficient in IFN type-I and II responses) and non-human primates (NHP). DENVax consists of a molecular clone of DENV-2 PDK53 and three chimeras, engineered to express the structural glycoproteins of DENV-1,-3, and -4. The AG129 mice and NHPs were pre-exposed (by intradermal or subcutaneous injection, respectively) to DENV-2 16681 or DENV-4 1086 and boosted by the same route with DENVax vaccine 42 and 60 days later, respectively. In both species, all animals infected with wt DENV-2 or wt DENV-4 mounted a strong neutralizing antibody response to the homologous virus and weak cross-reactive responses to the other DENV serotypes. In the presence of pre-existing immune responses to wt DENV-2 or wt DENV-4 there was no detectable viremia to any of the DENVax viruses following DENVax immunization. Analysis of the neutralizing antibody responses in individual animals following DENVax immunization showed that in both species the dominant neutralizing antibody specificity was against the DENV serotype to which pre-existing immunity had already been established. Interstingly pre-existing immunity to DENV-2 had a pronounced immunopotentiating effect on neutralizing antibody responses against all four serotypes. Overall, findings from this study suggest that pre-existing immunity has no detrimental effect on DENVax immunogenicity. Rather, neutralizing antibody responses to multiple DEN serotypes were enhanced. These preclinical studies support the safety and immunogenicity of DENVax administration in endemic countries; a Phase 2 clinical study directly addressing this issue is in progress.

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NEEDLE-FREE DELIVERY OF A CHIMERIC DENGUE-2 PDK-53-BASED TETRAVALENT VACCINE (DENVAX) IN NON-HUMAN PRIMATES

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To affect dengue vaccine delivery globally and in diverse clinical settings, an easy, practical and safe delivery method is required that enables the administration of vaccine in a needle-free fashion. Needle-free delivery approaches such as those using jet injectors are an attractive alternative to conventional needle and syringe injection. In this study we administered a tetravalent dengue vaccine, DENVax on day 0 and 60 by two routes of administration (subcutaneous; SC and intradermal; ID) using a novel,needle-free delivery device in non-human primates (NHP). Following SC priming with DENVax, the DENVax-2 component of the

tetravalent vaccine demonstrated a significant replication advantage over the other three chimeric DENVax components since only DENVax-2 RNA was detectable in the serum of vaccinated animals. In contrast, no viremia to any of the vaccine components was observed after ID administration. Analysis of the neutralizing antibody responses showed that vaccinated animals mounted primary and secondary neutralizing antibody responses to all four DENV serotypes. The DENVax-1,-2, and-3 vaccine components were the most immunogenic moieties of DENVax. In general, responses elicited by both routes were comparable but seroconversation rates following priming were superior via the SC route (100% for all four serotypes). In addition, the SC needle-free delivery of DENVax induced comparable neutralizing antibody responses to those induced by needle-and syringe. The protective efficacy of tetravalent DENVax was assessed against challenge with wild-type DENV-2. No viremia was observed in immune animals. In conclusion, the tetravalent DENVax vaccine administered with a needle-free vaccine delivery device has the potential to impact future mass vaccination campaigns by providing a safe, efficacious, and cost-effective dengue vaccine product targeted at the world market.

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PRECLINICAL TESTING OF A RECOMBINANT SUBUNIT VACCINE FOR DENGUE

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Dengue viruses are a major cause of morbidity and mortality throughout the tropics and subtropics. It is estimated that more than 120 countries currently have endemic dengue virus transmission and 55% of the world's population is at risk of infection. Globally, there are between 70-500 million infections each year, of which 2.1 million are clinically severe, resulting in more then 21,000 deaths annually. While a licensed dengue vaccine is not yet available, several vaccine candidates are currently being evaluated in clinical trials. Live attenuated vaccines for dengue have faced issues with interference between the four viral components. To overcome this issue Merck is evaluating a tetravalent recombinant subunit vaccine to protect individuals against dengue virus-induced disease. Preclinical studies have been conducted in non-human primates to evaluate the immunogenicity of tetravalent formulations prepared with different adjuvants and administered following different immunization schedules. The vaccine has been evaluated in both dengue naïve and experienced animals. These studies have shown the capacity of the recombinant proteins to induce durable, balanced tetravalent responses without evidence of interference. Efficacy as determined by protection from viremia following challenge with wild type viruses has also been demonstrated. Data from these preclinical non-human primate studies will be presented.

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THE DENGUE VACCINE INITIATIVE PROJECT IN COLOMBIA AND THAILAND: BURDEN OF DENGUE INFECTION IN CHILDREN AND ADULTS OF SANTA CRUZ COMUNA OF MEDELLIN AND BANG PHAE DISTRICT OF RATCHABURI PROVINCE

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Dengue infection is a major public health problem in both Colombia and Thailand, likely early adopters of dengue vaccines. Colombia experienced its largest epidemic with almost 157,000 DF cases and 217 deaths in 2010 and Thailand has reported provincial incidence rates up to 698/100,000 person years. In preparation for the upcoming dengue vaccine, the Dengue Vaccine Initiative is conducting epidemiological studies in Colombia and Thailand. The data from Ratchaburi province, Thailand and Medellin, Colombia will be used to estimate the burden of dengue infection in both adults and children, providing essential evidence for decision-making for vaccine introduction. DVI is conducting a passive facility-based surveillance complemented by a healthcare utilization survey (HUS) and a sero-survey to determine the burden of dengue in Bang Phae district of Ratchaburi province in Thailand and Santa Cruz comuna of Medellin in Colombia. In the surveillance, febrile patients between 1-55 years-of-age are evaluated for dengue infection. For the sero-survey, 2000 randomly selected residents are enrolled to estimate age-specific sero-conversion rate. From the HUS, we identify the proportion of febrile cases missed by the passive surveillance. Passive fever surveillances were launched in October and November, 2011 in Bang Phae Community Hospital and Santa Cruz Hospital, respectively. It has been a season of low caseload for both sites with only 173 and 77 subjects enrolled by March 2012, respectively. Thus far, we have 3 and 15 cases of acute dengue infection, including 3 primary and 12 secondary infections, examined using IgM ELISA in Bang Phae and Santa Cruz, respectively. In the sero-survey, 61% (n=1191) were found to be positive by IgG indirect ELISA among 1955 samples processed in Colombia. The first bleeding for the sero-survey in Thailand was just completed in mid-May, 2012. More data will be available for presentation by the end of 2012. The data generated, in addition to other economic, behavioral, market-demand collected in both sites, will be used to build comprehensive national investment cases of dengue vaccine in Thailand and Colombia. The investment cases will be used as models for other countries in the respective regions to facilitate accelerated development and introduction of safe and effective dengue vaccines.

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DENGUE VACCINE INITIATIVE: A MULTI-CENTER STUDY OF THE ECONOMIC BURDEN OF DENGUE INFECTION AND HOUSEHOLD WILLINGNESS TO PAY FOR DENGUE VACCINES

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As a dengue vaccine approach licensure, there is a need to examine the potential benefits of vaccine introduction. The Dengue Vaccine Initiative is conducting studies on dengue societal cost of illness (COI) and the private willingness to pay (WTP) for dengue vaccines in Ratchaburi province, Thailand; Medellin, Colombia; and Nha Trang, Vietnam. The data will be used to estimate the potential economic benefits of vaccination strategies, providing an improved evidence base for policymakers. The COI study estimates the direct/indirect costs associated with 200 dengue-confirmed cases at each site, both inpatient and outpatient. A survey instrument has been designed to collect a) the out-of-pocket costs for medicines, diagnostics, health service delivery, and transport and b) indirect cost due to productivity losses of patients and their care takers. Data will be collected during the first visit at treatment facilities, at 10-14 days post presentation, and at 28 days, if the patient is not fully recovered. The total COI per case will be calculated from the sum of out-of-pocket payments, indirect costs, and the facility treatment cost net of any patient co-payments. To estimate household demand and WTP for hypothetical vaccines against dengue infection, we administer a study questionnaire to 400 households at each site. Qualitative information regarding perceptions of dengue infection and the local need for dengue vaccines, and the perceived effectiveness of alternative prevention activities was collected during focus groups and pre-tests conducted in each site to refine the instrument. Respondents will be presented with one of five randomly assigned prices and asked how many vaccines they would purchase for the household and which family members would receive the vaccine. COI models will be developed to estimate the cost per dengue case based on severity and age. An economic private demand count model will estimate the average number of vaccines purchased per household as a function of vaccine price, efficacy, household perceptions of dengue severity and likelihood, as well as household socio-economic characteristics. Both parametric and non-parametric estimates of average WTP can provide information regarding the private benefits of vaccination. In parallel with epidemiological investigations conducted in the same areas, the package of results will provide an evidence for policy making for dengue vaccine introduction.

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INTENSIVE TRAINING IN MALARIA DIAGNOSIS TO STRENGTHEN THE MALARIA CONTROL PROGRAM IN ANGOLA AND MOZAMBIQUE

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Microscopic identification of *Plasmodium* spp. is the gold standard for the laboratory diagnosis of malaria. The identification of *Plasmodium* parasites to the species level can be determined by the examination of stained blood smears, but it relies on the technical expertise of the microscopist. Refresher training is essential to maintain and improve this expertise. We developed training tools and approaches to build training workshops in malaria diagnosis such as: 1-A digital training module with an archive of 800 digital images with different *Plasmodium* spp. presentations from clinical specimens obtained worldwide; 2- An archive of 150 microscope slides with thick and thin Giemsa-stained smears to enhance the practical exercises; 3- A training approach based on pre and post-tests comparison to verify the progress of participants. Each workshop consisted of examination of morphologic features of *P. falciparum*, *P. malariae*, *P. ovale* and *P. vivax* using digital images and stained slides in addition to the appropriate use of RDTs. The pre- and post- tests consisted of examination of 20 slides. Participants' scores were determined based on the percentage of slides that were identified correctly. We report the results obtained from intensive training in microscopy for diagnosis of malaria conducted in Angola and Mozambique between 2008 and 2011. To ascertain the short-term benefit of these trainings we analyzed pre- and post-test data from 120 laboratorians trained from 2008 and 2011. The data accumulated show that pre-test and post-test scores for Mozambique (N=61) were 50% and 80%, respectively. The results obtained in Angola were very similar (N=60) with the pre-test and post-test averages being 54% and 80%, respectively. We believe that intensive training on microscopic diagnosis of malaria should be a significant part of the malaria control programs in different countries.

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POSITIVE DEVIANCE: AN INNOVATIVE APPROACH TO IMPROVE MALARIA PREVENTION AND TREATMENT PRACTICES AMONG MOBILE AND MIGRANT WORKERS IN CAMBODIA

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Reaching mobile and migrant populations is one of the key strategies in the containment and elimination of artemisinin resistance in the Greater Mekong Subregion (GMS). Positive Deviance (PD) is an asset-based behaviour change approach with the underlying notion that every community has certain individuals (positive deviants or champions) whose malaria prevention and treatment practices result in better health outcomes than their neighbours. Malaria Consortium (MC) supported Cambodia's National Malaria Programme to pilot PD among residents and migrants in three villages in Sampov Loun district. The PD pilot aims to identify and promote good health seeking practices in both communities. The baseline survey conducted in Aug 2010 (n=309), suggested that knowledge about malaria and prevention were high in both communities but health-seeking behaviour for fever could be improved (residents 44.4%; migrant 33.3%), The PD process included 6 steps: pre-orientation meeting, community orientation, situation analysis, PD inquiry, participatory analysis, and community feedback. During the process, 13

in-depth interviews and 6 group discussions were conducted to identify the champions. For example, we identified a female migrant worker who never gets malaria by always sleeping under a bed net, wearing long sleeved clothes, covering her legs with a scarf while watching TV, and immediately going to the health centre when ill. All PD practices were shared with other community members through a 6 month PD-informed intervention which included training of volunteers, interactive health education sessions, role plays, art competitions and an advocacy seminar. An follow up survey is conducted in Mar 2012 (n=378) to better evaluate this intervention. Data entry and analysis is in progress and results will be presented in the ASTMH meeting, but preliminary results suggest that PD can serve as 1) a malaria intervention targeting migrants; 2) an alternative or supplementary method to deliver existing BCC/IEC interventions; and 3) an innovative model to promote community-based, bottom-up approaches.

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PARASITE-BASED MALARIA DIAGNOSIS: ARE HEALTH SYSTEMS IN UGANDA EQUIPPED TO IMPLEMENT THE POLICY?

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Effective coverage of parasite-based malaria diagnosis remains limited in malaria endemic countries. This study assessed the health systems capacity to absorb parasite-based malaria diagnosis (PMD) as an intervention at primary health care facilities in Uganda. In a cross sectional survey, using multi-stage cluster randomisation, level II (HCII) and III (HCIII) health facilities in 11 districts in Uganda were assessed for tools, skills, staff and infrastructure, and structures, systems and roles necessary for the implementing PMD. Health facilities: Out of the 125 heath centers (HC) evaluated, 64 (51%) were HCII and 49% were HC III. PMD was available at 30 (24%) of the HCs, microscopy in 18(30%) of the 61 HC IIIs the lowest level with laboratory facilities and RDTs in 12(20%) of all facilities surveyed. Three-months'-long stock-outs of oral and parental quinine were reported at 48% and 39% of the health facilities respectively. On average, half of the approved staff positions were vacant. All facilities had out-patient (OPD) registers, but did not uniformly capture vital mortality data. Health workers: Only 18%(131/730) of the recommended staff positions at the health centres were filled.. Out of the 131 health workers interviewed, 86 (66%) were nursing assistants. Only 47 (36%) of these health workers were sufficiently knowledgeable in managing severe malaria. Fifty six (43%) had received on-job training and supervision on malaria management within the 6 months prior to the survey. The top three reasons for referral were 1) poor response to treatment 66 (35%), 2) need for blood for transfusion 55(30%) 3) need intravenous fluids 31(17%). Overall, one-in-ten of the patients received adequate referral. Primary health care facilities had inadequate human and infrastructural capacity to effectively implement universal parasite-based malaria diagnosis. The priority capacity building needs were 1) training of health workers in fever management, 2) recruitment of qualified staff, 3) supply chain and stock management systems 4) referral and quality control systems.

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ANTIMALARIAL MARKETS AND TREATMENT-SEEKING BEHAVIOR FOR SUSPECTED MALARIA IN MYANMAR AND CAMBODIA: RESULTS FROM ANTIMALARIAL OUTLET AND HOUSEHOLD SURVEYS 2011-2012

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Artemisinin-resistant parasites were first detected around the Thai-Cambodia border, and there is evidence that resistance may be emerging in eastern Myanmar. Resistance to antimalarial drugs has historically spread from Southeast Asia to Africa, threatening recent global malaria control progress. Monitoring antimalarial markets and treatment-seeking behavior is key for informing and monitoring resistance containment strategies. The ACTwatch research program conducted nationally representative antimalarial outlet and household surveys in 2009 and 2011 in Cambodia. Data from these studies reflect changes in the antimalarial market and treatment-seeking behavior in the context of initiatives to improve case management coverage, such as the Village Malaria Worker program, as well as challenges to maintaining coverage due to stock-outs in the public and private sectors. Population Services International (PSI) conducted baseline household and private sector antimalarial outlet surveys in eastern Myanmar in 2011/2012. Results are representative of the private antimalarial market and treatment-seeking behavior in areas that comprise the Myanmar Artemisinin Resistance Containment (MARC) project, where partners are implementing resistance control strategies focused on replacing the widespread use of artemisinin monotherapy with ACT. Results from these studies and implications for improving coverage of appropriate case management and containing spread of artemisinin resistance will be discussed.

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AWARENESS ON THE AFFORDABLE MEDICINES FACILITY FOR MALARIA IN THE PRIVATE SECTOR IN GHANA

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The World Health Organization (WHO) estimates that about 40% of the world's population, mostly those in the Sub-Saharan Africa are at high risk of malaria. Malaria causes significant morbidity and mortality in Ghana. Artemisinin-based combination therapy is the recommended treatment for uncomplicated malaria but these medications are expensive making it inaccessible to people who need them. The Affordable Medicines Facility for Malaria (AMFm) is a mechanism to subsidize ACTs to increase access to Artemisinin-based Combination Therapy (ACTs) for treatment of malaria. To increase awareness of ACTS and acceptability of the initiative, an intensified Behavioural Change Communication and training of health workers was rolled out nationwide. The objective of this study is to determine the knowledge of private pharmaceutical outlets on the Affordable Medicines Facility for malaria (AMFm) and on the co-paid ACTs .One hundred and fifty one (151) pharmacies and five hundred and ninety one (591) LCS shops were randomly selected from the most urbanized and least urbanized districts across the entire country and monitored for three consecutive months. In-depth structured interview, using a mix of open-ended and closed questions, were used to determine health workers knowledge on the AMFm and the ACTs. Six months after commencement of the communication campaign on AMFm and ACTs, 95.4% respondents surveyed in the Licensed Chemical Shops and Pharmacies interviewed were aware of the AMFm initiative and the co-paid ACTs. The awareness level was higher in the urban areas (76%) than in the rural areas (33%) (p-value < 0.05). Awareness on the AMFm and on the co-paid ACTs

was higher in the Licensed Chemical Stores (98.5%) as compared to the Pharmacies (81%). Awareness of the AMFm has become high and knowledge of the co-paid ACTs has been increased due to the intensive campaign that were undertaken, thereby making demand for the ACTs with the green leaf logo very high in Ghana.

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MONITORING ANTIMALARIAL DRUG USE IN RELATION TO NATIONAL DRUG POLICY

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The majority of malaria-related deaths occur in sub-Saharan Africa and two of the most vulnerable groups identified are children under the age of five years and pregnant women. Historically, malaria has evolved resistance to the main first line antimalarial drugs, such as chloroquine and sulphadoxine-pyrimethamine. This has necessitated changes in national drug policies in African countries over the past decade towards artemisinin combination therapies (ACTs). The length of time between policy changes and their subsequent implementation, following the emergence of resistance, directly affects public health because of the mortality rates associated with a lower treatment efficacy. In this work, we have extracted data on treatment taken by children aged under five as a response to fever to estimate drug use and pressure on the African continent and describe the relationship with national policy changes. The work has been limited to chloroquine and sulphadoxine-pyrimethamine, as sufficient recent data on ACTs were unavailable. We interrogated databases from Demographic Health Surveys (DHS) and Multiple Indicator Cluster Survey (MICS) from 43 countries in Africa over the period 1993-2010. Based on the 99 studies that were included in our analysis over this period we have fitted logistic regression models, for chloroquine and sulphadoxine-pyrimethamine, for the proportion of fever cases treated with each drug over time (measured since policy change away from the drug), with country included as a random effect. We find that while drug use post policy change does exhibit a downward trend, there is still significant usage of these outdated drugs. We discuss the implications of drug use and its relationship to national policy for effective care of malaria, and for managing resistance.

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SURVEILLANCE IN THE SOUTH PACIFIC, THE ESTABLISHMENT OF BASELINE DATA AND MONITORING FOR CHANGE IN MALARIA DRUG RESISTANCE PROFILES

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Vanuatu and the Solomon Islands (SI) are progressing towards malaria elimination. Artemether-lumefantrine (Coartem™), an artemisinin combination therapy (ACT), was introduced as first line therapy for both *falciparum* and *vivax* malaria in these countries in 2008, and forms the cornerstone of the malaria control/elimination programs in both countries. Reports of artemisinin resistance developing in SE Asia demonstrate that ongoing surveillance is essential to detect and monitor for artemisinin resistance. Additionally, these countries are among the few where ACT is used for treatment of both *falciparum* and *vivax* malaria allowing for detection of potential changes in resistance profiles of both species without CQ and SP pressure. Our aim is to obtain base-line data during Coartem introduction allowing for future detection of changes in drug resistance profiles. Samples collected from baseline epidemiological surveys in Tafea Province, Vanuatu and Temotu Province, SI along with samples from a Coartem efficacy study conducted in Malaita Province, SI were investigated for the prevalence, distribution and origins of drug resistant *Plasmodium* parasites by examining sequence polymorphisms within known drug resistant markers (*pfcrt, pfdhfr, pfdhps, pvdhfr* and *pvdhps). Pfcrt* analysis revealed 100% (Tafea and Malaita) and 98% (Temotu) of parasites carried the K76T mutation indicative of CQR; microsatellites flanking *pfcrt* are similar to those in Papua New Guinea suggesting these CQR parasites share a common ancestry. Variations in *pfcrt* genotypes were detected between and within these two countries, even on smaller islands with limited populations. In Vanuatu three *pvdhfr* alleles were observed with the majority containing the double polymorphism, 58R/117T. Similarly, *pfdhfr* revealed a dominance of the double polymorphism 59R/108N. In Malaita the most common *pvdhfr* allele was the quad mutant 57L/58R/61M/117T. Unlike the variability exhibited in the *pvdhfr* gene, 100% of samples possessed the drug sensitive *pvdhps* allele 382S/383A/512K/553A/585V.

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DEVELOPMENT OF GENOMICS RESOURCES FOR *BABESIA MICROTI*, AN EMERGING INFECTIOUS DISEASE AGENT IN THE UNITED STATES

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Babesia microti is the principal cause of human babesiosis and one of the most common transfusion-transmitted pathogens in the United States. The parasite has a worldwide distribution and has been cited as an emerging health threat in the United States. *B. microti* is primarily transmitted to humans by the tick vector, *Ixodes scapularis*, but transmission also occurs perinatally and through blood transfusion. We have sequenced and annotated the genome of a clinical isolate of *B. microti*. Comparative genomic studies with other protozoa reveal that *B. microti* has the smallest nuclear genome among all apicomplexa with three chromosomes encoding ~3,500 polypeptides, including species-specific genes and gene family expansions. Genome-wide reconstruction of functional networks revealed the minimal metabolic requirements for intraerythrocytic parasitism by protozoan parasites. Furthermore, unlike all other Apicomplexa, its mitochondrial genome is circular. This study resulted in the identification of several targets suitable for diagnosis and treatment of human babesiosis. Remarkably, genome-wide phylogenetic analyses indicate that *B. microti* is significantly distant from all species of Babesidae and Theileridae and defines a new clade in the phylum Apicomplexa. Efforts are now underway to sequence the genome and transcriptome of six new isolates of *B. microti*. These studies will inform on *B. microti* pathogenesis, genetic diversity, evolution and virulence and will open new avenues for future design of improved diagnostic and treatment strategies.

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AGE-DEPENDENT GENETIC ASSOCIATIONS WITH *CRYPTOSPORIDIUM* INFECTION IN BANGLADESHI CHILDREN

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Infection by *Cryptosporidium*, a protozoan parasite, is one of the major causes of diarrhea in children globally. Up to 12% of diarrheal disease in children less than 2 years of age in developing countries may be attributed to *Cryptosporidium,* with more severe outcomes in malnourished children. Infection early in childhood can lead to stunting as well as impaired cognitive development. The human immune response to *Cryptosporidium* has not been well-characterized, especially in young children where the burden is the highest. Prior studies demonstrated the association of HLA Class I and II alleles with cryptosporidiosis in young children, suggesting a genetic involvement in the host response to *Cryptosporidium* infection. To identify host genetic factors that may play a role in susceptibility to infection, we conducted a genome-wide association study (GWAS) in 374 children from Dhaka, Bangladesh participating in a birth cohort using 6.6 million single nucleotide polymorphisms imputed to 1000 Genomes. Diarrheal stool samples were collected less than 24 hours after a report of a new diarrheal episode and tested for the presence of *Cryptosporidium*. Of these 374 children, 58 had cryptosporidiosis within the first year of life. For the 350 children with 2 years of follow-up, 99 had at least one *Cryptosporidium* infection. Associations were calculated across the human genome using an additive genetic model. Comparing children with or without infection within the first year of life, two regions reached a genome-wide significance: 1q32 ($p=1.5x10^{-7}$) and 11q24 ($p=4.6x10^{-7}$). An additional suggestive signal was found in chromosomal region 5p15 with a p-value of $8.7x10^{-7}$. We extended the association to compare children with or without infection in the first two years of life and two regions showed genome-wide significance: 2p21 ($p=3.5x10^{-7}$) and 4p14 ($p=3.7x10^{-7}$). This study suggests that host genetic factors may be important for susceptibility to *Cryptosporidium* infection in early life and these host genes may be age-dependent.

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BREAST MILK IGA AGAINST *CRYPTOSPORIDIUM* PROTECTS BANGLADESHI INFANTS FROM INFECTION

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Cryptosporidium diarrhea is a major contributor to morbidity among infants in the developing world. There are limited therapeutic options for young children, and there is currently no vaccine available. Here we investigated whether *Cryptosporidium*-specific breast milk IgA could protect infants from *Cryptosporidium* infection. A longitudinal cohort study of 226 infants was followed by twice-weekly home visits from birth through the first year of life in a Dhaka slum community. Diarrheal and non-diarrheal monthly surveillance stools were collected from each infant and tested for *Cryptosporidium* using qPCR. Breast milk samples were collected from each mother in the first month after birth and tested for IgA against *Cryptosporidium* oocysts. By 12 months of age, 40% of children had been infected with *Cryptosporidium*. Infants whose mothers had high levels of anti-Cryptosporidium breast milk IgA had a lower risk of *Cryptosporidium* infection (defined as a diarrheal or monthly stool sample positive by PCR for Cryptosporidium, p=0.021) and had a higher chance of survival free of *Cryptosporidium* infection (p = 0.039). This study is the first to report that breast milk antibodies can be protective against *Cryptosporidium* infection. This is a significant finding, as immunity against this parasite has been thought to be primarily cell-mediated. Our findings suggest that passive immunity may have important implications for prevention of *Cryptosporidium* infection in breastfed infants as well as for treatment of fulminant disease in immunocompromised adults.

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DISTRIBUTION OF DRUG RESISTANCE AND ITS GENETIC BASIS IN GLOBAL ISOLATES OF *TRICHOMONAS VAGINALIS*

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The emergence of antibiotic-resistant pathogens remains one of the most challenging problems in health care. Antibiotics influence microbial communities in the human body by changing microbial ecology, with the potential to increase resistance. *Trichomonas vaginalis* is the most prevalent non-viral sexually transmitted pathogen in the world. Although trichomoniasis had long been regarded as a sexually transmitted infection of minor importance, evidence implicates *T. vaginalis* as a contributor to a variety of adverse outcomes such as increased transmission of HIV, cervical cancer and adverse pregnancy outcomes. The parasite is commonly treated with the nitroimidazole group of antiprotozoal agents but ~5% of isolates display drug resistance. Analysis of global genetic variability in *T. vaginalis* isolates indicates that resistant isolates cluster within one of two primary subpopulations, suggesting that it will be possible to isolate genes and pathways that are involved in drug resistance. In order to address the prevalence of drug resistance phenotype in different geographical regions, we have performed resistance assays with two of the most commonly used drugs (metronidazole and tinidazole) in 187 *T. vaginalis* isolates from seven geographical regions to: (1) confirm their drug susceptibility status and (2) determine if the resistance is heritable. Our analysis of clonally identical genotypes based on 21 *T. vaginalis* microsatellite loci in each geographical region did not identify more than three identical clones, suggesting the necessity for larger genetic screens. We are also developing genome wide screens to identify the genetic loci responsible for drug resistance, which will allow us to test if the same genes are responsible for drug resistance in different geographical regions. Comparison of *T. vaginalis* natural isolates exhibiting a range of drug susceptibilities is an important step towards understanding how the parasite modifies its molecular makeup with permanent genetic changes to overcome the challenge of drug pressure.

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IMPROVING THERAPEUTICS FOR THE TREATMENT OF CRYPTOSPORIDIOSIS USING HIGH THROUGHPUT METHODS

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Cryptosporidium parvum and *Cryptosporidium hominis*, the most common etiological agents of cryptosporidiosis, are the leading cause of waterborne diarrheal outbreaks in the United States and second most common cause (after rotavirus) of pediatric infectious diarrhea in Africa and Asia. Susceptibility to the parasite has been correlated with nutritional status in children, and chronic infection has been associated with decreased functional status. Infection is often self-limited in healthy individuals, but results in chronic, fulminant, and often fatal illness in immunocompromised populations. Nitazoxanide, the current therapeutic gold standard, is effective at reducing the duration of infection in immunocompetent individuals, but is no more efficacious than placebo in immunocompromised populations. The high morbidity and mortality in pediatric and AIDS patients, combined with unavailability of highly

active antiretroviral therapy makes cryptosporidiosis a severe public health problem in the developing world. While excessive cost makes the *de novo* development of anti-cryptospordial medications nearly impossible, drug repurposing may provide a more feasible solution. The discovery of new uses for already approved compounds with demonstrated safety records facilitates the efficient entry of candidate medications into clinical efficacy trials. We have developed a robust (Z' score= 0.27-0.41) cell-based high throughput screening (HTS) platform to test inhibitors of *Cryptosporidium parvum* and conduct follow-up testing. We have successfully screened the NIH Clinical Collection, a compound library of 727 approved drugs and experimental drug-like compounds, and identified 11 compounds with ≥ 90% inhibition, and 25 compounds exhibiting ≥ 80% inhibition *in vitro*. 20 compounds were identified as potential leads and repurchased for follow-up testing including the determination of IC_{50} and TD_{50}, potential for synergy, and to determine whether the drugs employ static or cidal mechanisms of parasite killing in order to inform prioritization for further *in vivo* follow up.

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WHOLE-GENOME CAPTURE OF *THEILERIA PARVA*, AN APICOMPLEXAN PARASITE OF CATTLE IN SUB-SAHARAN AFRICA

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East Coast fever (ECF), which occurs in eastern, southern and central Africa, is an acute fatal disease of cattle caused by the tick-transmitted intracellular apicomplexan pathogen *Theileria parva.* Every year ECF kills >1million cattle, the primary source of food and income for many smallholder African farmers. The "infection-and-treatment" immunization method (ITM) recently adopted in areas of sub-Saharan Africa induces long-term immunity based on CD8+ T-cell responses, but has significant logistical and economic drawbacks, making the development of an effective recombinant vaccine a high priority. In the last few years reverse vaccinology has emerged as a primary approach to identify putative vaccine antigens from genome sequence data. However, the application of reverse vaccinology to eukaryotic pathogens still presents several fundamental challenges. In the case of *T. parva*, those challenges start with the isolation of parasite DNA for genome sequencing. *T. parva* piroplasm DNA can be obtained from bovine blood in sufficient quantity for genome sequencing but results in the sacrifice of the bovine host. Here we report the successful capture and sequence of *T. parva* genomic DNA from a *T. parva*-infected lymphocyte cell line. The capture probe set was designed to cover 97% of the 8 Mb genome of the *T. parva* Muguga strain, including 98% of its annotated genes. The fragments captured from both Illumina and 454 libraries built from infected lymphocyte DNA mapped to 97-98% of the *T. parva* Muguga genome. Unmapped reads totaled less than 25% of the captured reads, establishing the selective preference of *T. parva* DNA over that of the host. Ongoing experiments will determine the success of this approach to capture *T. parva* genomic DNA from lymphocyte cell lines infected with divergent isolates of this species. These results demonstrate the feasibility of whole-genome sequence capture to isolate parasite DNA from a mix of parasite and host DNA. Furthermore, the results are directly applicable to a variety of human intracellular pathogens of similar genome size and complexity, including several apicomplexan parasites.

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THE BURDEN OF CRYPTOSPORIDIOSIS AND ITS EFFECT ON GROWTH IN A BIRTH COHORT OF CHILDREN IN AN URBAN SLUM OF SOUTH INDIA

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Cryptosporidiosis is a major cause of diarrheal disease in developing countries, predominantly affecting children under 5 years of age. Cryptosporidiosis in early childhood is reported to affect growth and development. To estimate the burden of cryptosporidiosis and its effect on growth preliminary data from an ongoing birth cohort study in an urban slum of South India were analyzed. Children (N=413) were followed biweekly from birth to 18 months of age to assess diarrhea and other morbidities. Stool samples were collected every 2 weeks and during a diarrheal episode (N=17,875) and tested for *Cryptosporidium* spp. by PCR. Anthropometric (height and weight) measurements were obtained monthly. Acute (wasting, WHZ < - 2 SD) and chronic (stunting, HAZ < - 2 SD) malnutrition at 18 months of age was assessed using the WHO Child Growth Standards 2006. The effect of symptomatic and asymptomatic cryptosporidiosis on stunting and wasting was assessed using a logistic regression model adjusted for crowding (>5 members/household), presence of older siblings, exclusive breastfeeding up to 6 months, low birth weight (<2500 grams, LBW), gender, religion, and socioeconomic status (SES). By 18 months, 190 (46%) children experienced one or more episodes of cryptosporidiosis. In 121 (63.8%) children who had only asymptomatic infection/s and 69 (36.2%), children who had at least one symptomatic infection the proportion with repeated episodes were similar (20 (16.6%) and 16 (23.2%), p=0.25). The mean (95% CI) ages at first asymptomatic and symptomatic infections were also similar (8.4 (7.2-9.6) vs. 8.8 (8.2-9.4) months, p=0.54). At 18 months, 122 (30%) children had stunting and 54 (13%) had wasting. Both symptomatic (OR: 1.9 (1.1-3.5), p=0.03) and asymptomatic (OR: 1.7 (1.1 -3), p =0.02) infections had a significant effect on stunting. LBW (OR = 3.5 (2 -6.3, p <0.001) and SES (OR =1.6 (1- 2.8), p= 0.04) were predictors for stunting. In children with LBW, symptomatic infection was predictive for stunting (OR= 3.9 (1 -15), p=0.04). Acute malnutrition was more pronounced in boys (p=0.01). These findings indicate that cryptosporidiosis was significantly associated with chronic malnutrition by 18 months of age.

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DIFFERENTIAL ANTI-GLYCAN ANTIBODY RESPONSES IN *SCHISTOSOMA MANSONI*-INFECTED CHILDREN AND ADULTS STUDIED BY SHOTGUN GLYCAN MICROARRAY

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Schistosomiasis is a chronic and potentially deadly parasitic disease that affects millions of people in (sub)tropical areas. Infected individuals do acquire immunity to *Schistosoma*, but this takes many years of exposure, multiple infections and treatments, and maturation of the immune system. Therefore, children are more susceptible to re-infection than older children and adults. This age-dependent immunity or susceptibility to re-infection after treatment has been shown to be based on antibody as well as T cell responses. Most antibodies generated during *Schistosoma* infection are directed against the abundant glycans expressed by the parasite, of which an unidentified subset appears to be protective. The structure of most of the glycan epitopes recognized by antibodies is however unknown. The interaction of antibodies with glycans can be studied efficiently and quantitatively using glycan microarray approaches in which small amounts of a large number of glycans are presented on a

glass slide. We have generated a shotgun glycan microarray containing natural N-glycan and lipid glycan fractions derived from 4 different life stages of *S. mansoni* and applied this array to the analysis of IgG and IgM serum antibodies in a selection of sera from children and adults living in an endemic area. For the first time, we have analyzed the anti-glycan antibody responses in *Schistosoma* infection against many different glycan elements simultaneously. The antibody responses against many of the *Schistosoma* derived N-glycan and lipid glycan fractions are on average higher in children than in adults and are dominated by IgM and may reflect differences in age or differences in length of exposure or infection. We have shown that the shotgun glycan microarray approach has strong potency to study antibody response profiles and allows the definition of patient groups as well as glycan element clusters to which antibody responses are generated and can be applied to select potential glycan vaccine elements.

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T REGULATORY LYMPHOCYTES AND IMMUNE RESPONSES TO SCHISTOSOME ANTIGENIC PREPARATIONS BY INDIVIDUALS WITH EITHER *SCHISTOSOMA MANSONI* "RECENT RE-INFECTIONS" OR "CHRONIC" INFECTIONS

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Patients chronically infected with *Schistosoma mansoni* exhibit high levels of circulating regulatory T cells (Treg), which are associated with immune regulation in murine experimental schistosomiasis. The functional role of Treg in human schistosomiasis has not yet been established, but it is hypothesized that they act to reduce proliferation and cytokine production by schistosome specific responder T lymphocytes upon appropriate encounters with schistosome antigens. We find that men with infections of 2 years or longer ("chronic") have higher levels of circulating CD4+/CD25hi Treg cells than men re-infected 8 months or less prior to being studied ("recent re-infections"). We have also phenotyped lymphocytes from these subjects using monoclonal antibodies against FoxP3, CD127, CD45RA, CD45RO, CD357 (GITR), HLA-DR, and CTLA4 and find that CD25hi and CD45RAneg parameters best characterize the FoxP3+ Treg population we are studying. Using a BrdU ELISA assay to measure Peripheral Blood Mononuclear Cell (PBMC) proliferation to Soluble Egg Antigens (SEA) or Soluble Worm Antigenic Preparation (SWAP), we see highly variable responses by individuals and by the two groups. Men with "chronic" infections with low PBMC responses to SWAP have increased responsiveness upon removal of their CD25hi/Treg populations using anti-CD25 magnetic beads, indicating that Treg play a regulatory role in their immune response to SWAP. We also find that two populations of men with "chronic" infections with different exposure histories have distinct Treg profiles. Men working as sand harvesters, most of whom were exposed to schistosomiasis from 2-4 years of age and were likely born of *S. mansoni*-infected mothers, have higher levels of circulating CD4+/CD25hi Treg cells than car washers, who may have only been exposed to infection in their adult years. These data provide new immunological insights into immunoregulation during human schistosomiasis and suggest possible further differences in the immunology of schistosomiasis infections based on their duration and exposure histories.

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A ROBUST AND REPRODUCIBLE PROCESS FOR PRODUCTION OF A SCHISTOSOMIASIS VACCINE

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Schistosomiasis causes significant morbidity and mortality in the developing world with recent studies indicating that the geographic extent and burden of the disease is higher than the official estimates. Although, Schistosomiasis is a treatable infection, the current treatments of choice do not provide an optimal strategy for controlling the disease. The high rate of post-treatment reinfection has made obvious the need for new approaches, such as vaccination, to complement the existing treatment initiatives. The increase in information regarding the mechanisms of immunity for Schistosomiasis infection has indicated that surface antigens may be effective vaccine candidates. Of this handful of proteins, some have already been shown to have potential as recombinant vaccines against Schistosomiasis at a pre-clinical level. In particular, the tetraspanin family of integral membrane proteins, highly abundant in the parasite tegument, has been shown to correlate with protective immunity in a mouse vaccine model, suggesting that this family of membrane proteins offers promise as a Schistosomiasis vaccine. The Pichia codon optimized DNA sequence of the extracellular domain of Sm-TSP-2 was synthesized and cloned into the Pichia expression vector pPinkα-HC. Here we describe the process development that led to the GMP manufacturing of one of the lead candidate antigens Sm-TSP-2 at a 20 L scale fermentation, and its formulation for phase I clinical trials. Throughout the process we confirmed the yield of recovery, the purity, and the integrity of the recombinant protein, as well as a comprehensive biophysical characterization of the protein itself.

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TARGETING SCHISTOSOME CERCARIAL PROTEASE FOR VACCINE DEVELOPMENT

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Parasitic blood flukes of the genus *Schistosoma* cause schistosomiasis, the most important helminthic infection in terms of morbidity and mortality in developing countries. Potential resistance to praziquantel, the only drug currently used to treat the disease, has already raised serious concerns about the necessity to discover and develop new drugs against schistosomes. The development of a subunit vaccine for schistosomiasis would also be a key step for disease control. Schistosome larvae (cercariae) are able to directly penetrate host skin facilitated by secretion of proteases from acetabular cells of cercariae in response to skin lipids. Recent proteomic analyses of secretions identified an S1A serine peptidase termed cercarial elastase (aKa or SmCE cercarial protease) as one of the most abundant proteins released by *Schistosoma mansoni.* SmCE is involved in both skin invasion and immune evasion. Preliminary work has shown that skin invasion by cercarie could be inhibited by serine protease inhibtors. To validate SmCE as a vaccine candidate, a proof of principle study will use ecotin (*Escherichia coli* serine protease inhibitor) as a model for protease directed antibodies. To apply this inhibitor to *ex vivo* skin, before exposure to cercariae, we are using a nanopatch technology with microneedles ensuring the delivery of ecotin to the epidermal layer of skin. In parallel, we are carrying out a second proof of principle study based on antibody-based inhibitors. We will "pan" SmCE against a diversity antibody library composed of biased Fab fragments designed to inhibit serine proteases.

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IMMUNO-MODULATION OF HUMAN IMMUNE RESPONSES DURING SCHISTOSOME INFECTION AND CONSEQUENCES OF HELMINTH TREATMENT PROGRAMS

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We have been involved in studies evaluating the need, safety and efficacy of treatment of paediatric schistosome infection. The results of these studies were recently published in a World Health Organisation document where we and others recommended that schistosome control programmes be extended to include pre-school children. The overall short- and long-term consequences of this recommendation need to be evaluated in light of experimental and field studies suggesting that helminth infection modulates immune responses directed against unrelated antigens. The factors affecting the magnitude and dynamics of the modulation of these immune responses in helminth-exposed people are still poorly understood. Thus, alongside the paediatric schistosome studies, we have been characterising the relationship between infection with the helminth *Schistosoma haematobium* and immune responses to unrelated antigens, including common allergens (house dust mite), self-antigens (nuclear antigens) and antigen from other pathogens *(Plasmodium falciparum* vaccine candidates). These immune responses have potentially important consequences for clinical allergy, autoimmune disease and malaria vaccine efficacy. We have also been conducting mechanistic studies involved in helminth-related immuno-modulation through comparative human studies. The studies, all conducted in the same two populations have provided significant insight into factors affecting host immune responses to different antigens during helminth infection and the effects of antihelminthic treatment on these responses across a wide age range of people with different histories of schistosome infection.

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REPEATED *SCHISTOSOMA JAPONICUM* INFECTIONS IN A SUBSET OF INDIVIDUALS FOLLOWING TREATMENT

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It has been shown that, at any point in time, a few individuals may be responsible for disproportionate transmission of infectious pathogens. However, for disease systems where reinfection is possible, are superspreaders the same individuals over time? To answer this question, we examined the incidence of repeated *Schistosoma japonicum* infections in two longitudinal cohorts in Sichuan, China. In each cohort, participants were tested for infection and treated with praziquantel at baseline, then followed prospectively and tested for infection two more times over a period of six (Cohort 1) and three (Cohort 2) years. Water contact behaviors were assessed and *S. japonicum* infections were promptly treated with praziquantel. *S. japonicum* infection prevalence at baseline was 47% (mean infection intensity 46 EPG) and 11% (mean infection intensity 2.6 EPG) in cohorts 1 and 2, respectively. The incidence of two consecutive infections was 1.5 times higher than expected in cohort 1 and 5.8 times higher than expected in cohort 2 ($p<0.001$ in both cohorts). As it is possible that individuals who are repeatedly infected with *S. japonicum* are the most highly exposed individuals in the population, we additionally predicted the expected incidence of repeated infection accounting potential exposure to cercariae-contaminated water sources using a data-adaptive, machine learning algorithm. The incidence of repeated infections was 1.3 ($p=0.013$) and 2.1 ($p<0.001$) times greater than expected in cohorts 1 and 2, respectively, accounting for host exposure. The clustering of infections within a limited number of hosts suggests infected individuals may be appropriate targets for intervention and surveillance. Moreover, fact that the repeated observation of schistosomiasis in the same individuals cannot fully be explained by host exposure suggests some individuals may be more susceptible to *S. japonicum* infection than their peers or, alternatively, these individuals do not, in fact have new infections, but have residual, uncured infections that persist despite treatment.

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ISOLATED AND PERSISTENT INTESTINAL SCHISTOSOMIASIS FOCI IN WESTERN PROVINCE OF BURKINA FASO

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The national schistosomiasis and soil transmitted helminthes control program in Burkina Faso implements mass drug treatment for underserved communities at risk for schistosomiasis since 2004. Urinary schistosomiaisis is endemic in all the districts of the country but intestinal schistosomiasis is restricted to the Dafra district in the Western province. We reviewed data collected in the village of Panamasso in the Dafra district from 2008 to 2012. The village is located in a humid area with high level of rainfalls favoring and sustaining ponds and small perennial water bodies. There is a tributary of the river *''We"* that spans through the village and constitutes the main source of water for domestic use for the 1427 inhabitants. The district has the perfect ecology, climate and freshwater to harbor snails of Biomphalaria genius, the intermediate host for *Schistosoma mansoni*. The communities depend on the water from the *''We"* river for various daily usage. Fishing is a major occupation sometimes creating itinerant groups that move and settle following fishing zones along the river. Risk factors of *S. mansoni* infection also include the total absence of even traditional latrines in the village and defecation in open area along the river and stagnant bodies of water within the village. The prevalence of *S. mansoni* was 30% at the onset of the program and increased yearly since 2008 reaching 38.54% in 2012 despite satisfactory PZQ treatment coverage of over 80%. Heavy intensity of infection has progressed from 8 % in 2008 to 37% in 2012 for people with > 400 eggs/gram. This review shows that in settings where the prevalence remains high, Schistosomiasis treatment frequency may need to be augmented to efficiently deal with *S. mansoni* transmission.

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ISOLATION OF *SALMONELLA SP.* FROM A *PTEROPUS GIGANTEUS* FRUIT BAT IN BANGLADESH

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Typhoid fever causes approximately 21 million cases and 0.2 million deaths worldwide annually with the highest incidence in South-East Asia. The etiologic agent of Typhoid Fever, Salmonella Typhi, is considered a host-adapted pathogen of humans. However, researchers identified that fruit bats (*Pteropus rufus*) were carriers of Salmonella Typhi in Madagascar in 1973. Based on this study in Madagascar, we conducted a cross-sectional study in three ongoing Nipah surveillance areas in Bangladesh to sample native fruit bats (*Pteropus giganteus*) for the presence of Salmonella Typhi and other Salmonella serotypes. From February to June 2010 we captured 302 Pteropus fruit bats, collected rectal swabs and placed them in Cary Blair media before transporting them to the Clinical Microbiology laboratory of icddr,b within 48 hours. Laboratory technicians used MacConkey agar, S-S agar and Selenite broth for isolation and S-S agar for sub-culture. We sent the isolate to the Enteric Diseases Laboratory Branch, CDC for serotyping. No Salmonella Typhi was recovered from any of the sampled bats. We recovered a single isolate of Salmonella of Group C1 from one juvenile female *P. giganteus* bat (prevalence 0.33%, 95% confidence interval 0.008%, 1.8%) from Faridpur district. The Enteric

Diseases Laboratory Branch, CDC identified this as Salmonella Virchow. Though we did not find any evidence of Salmonella Typhi, this does not exclude the possibility that Salmonella Typhi could be carried at least occasionally by *Pteropus* bats in Bangladesh. Salmonella serotype Virchow has not been previously reported from *Pteropus* bats. Salmonella Virchow had been isolated from humans in Bangladesh from two clinics. This bat may have become infected with Salmonella by drinking surface water contaminated with human feces. Since Salmonella serotype Virchow and other serotypes can be carried by bats, Pteropus bats may be involved in chains of salmonella infection that ultimately affect humans.

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EVALUATION OF TUBEX® TF, A RAPID DIAGNOSTIC TEST FOR TYPHOID FEVER, IN THE MIDST OF A LARGE, URBAN TYPHOID FEVER OUTBREAK - HARARE, ZIMBABWE 2011-2012

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Salmonella enterica serovar Typhi (*S.* Typhi) causes an estimated 22 million cases of typhoid fever and 216,000 deaths worldwide annually. We assessed the value of a rapid diagnostic test compared with blood culture during a large outbreak of typhoid fever in Harare, Zimbabwe. Suspected cases of typhoid fever were defined as persons who lived in or visited Harare since October 1, 2011, with ≥3 days of fever and one or more of the following symptoms: malaise, headache, vomiting, diarrhea, constipation, or cough. As of March 21, 2012, 3,932 suspected cases, including 1,857 hospitalizations and 2 deaths were reported. Fifty-two cases were confirmed by blood or stool culture. Cases ranged in age from 1-88 years (median 16 years); 54% were female. Cases were predominantly from the high-density suburbs of Kuwadzana (1,824) and Dzivaresekwa (919). The pulsed field gel electrophoresis pattern for seven of eight isolates was indistinguishable from that of two isolates from an outbreak in Zimbabwe in 2009 and from isolates from Malawi and Tanzania. We evaluated a rapid serum IgM antibody diagnostic test for typhoid fever, TUBEX® TF, compared to standard blood culture. Patients were enrolled at two facilities: a primary care polyclinic in the high-density Kuwadzana suburb of Harare and Beatrice Road Infectious Disease Hospital which provides inpatient care across Harare. Patients included in the analysis were ≥1 year old, residents of Zimbabwe, had a fever ≥38°C at time of enrollment and at least one of the symptoms noted above. As of April 2012, *S.* Typhi was recovered from 7 of 103 patients by blood culture; 70 cultures were negative and 26 were pending. To date, 13 serum samples, including 6 from culture-confirmed patients, were positive; 32 were negative, 31 were pending and 27 were excluded because of gross hemolysis. Sample collection is ongoing. In this large outbreak, preliminary results from TUBEX® TF testing correlated with blood culture results suggesting it might be a useful adjunct for clinical management and surveillance.

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IDENTIFICATION OF *IN VIVO*-INDUCED BACTERIAL PROTEINS DURING HUMAN INFECTION WITH *SALMONELLA ENTERICA* SEROTYPE PARATYPHI A

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We applied an immunoscreening technique, *In Vivo*-Induced Antigen Technology (IVIAT), to identify immunogenic bacterial proteins expressed in humans bacteremic with *Salmonella enterica* serotype Paratyphi A, the cause of paratyphoid fever. We were able to assign a functional classification to 15 of 20 proteins identified by IVIAT. Of these 15, the majority represent proteins with known or potential roles in the pathogenesis of *S. enterica*. These include proteins implicated in fimbrial structure, antimicrobial resistance, bacterial motility, heavy metal transport, bacterial adhesion, sugar transport, and anaerobic respiration. The five remaining antigens represent proteins with unknown functions. Transcripts for 15 of the 20 genes were previously identified in the blood of humans bacteremic with *S.* Paratyphi A or *S.* Typhi. We confirmed increased expression of mRNAs expressed by genes identified by IVIAT by quantitative-PCR. Of the 20 identified antigens, we examined differential immunoreactivities in acute versus convalescent phase human serum samples for five antigens; these five included BcfA, YcfL, StcD, Gp19, and one antigen of unknown function encoded by SPA0489. BcfA is a fimbrial subunit involved in bacterial adhesion with no homolog in *E. coli*. YcfL is a putative periplasmic lipoprotein. StcD is a putative exported protein. Gp19 is a lysozyme that facilitates biosynthesis of the cell wall during cell division. SPA0489 encodes a protein of unknown function that is unique to *S.* Paratyphi A. Since *S.* Paratyphi A is a human restricted pathogen, there are limited data on host-pathogen interactions. Additionally, there is currently no commercially available vaccine for *S.* Paratyphi A and diagnostic assays lack sensitivity and specificity. *S.* Paratyphi A antigens identified by IVIAT warrant further evaluation for their contributions to pathogenesis, and may have diagnostic, therapeutic, or preventive uses.

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SMALL SCALE POULTRY FARMING AND ZOONOTIC TRANSMISSION OF ANTIBIOTIC RESISTANCE IN RURAL ECUADOR

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Poultry farming is promoted for international development because poultry are inexpensive, an efficient source of protein, and have few associated cultural beliefs. While poultry farming offers a promising economic strategy, there is a risk of development of antibiotic resistant bacteria. Previous studies have reported shared resistant strains between humans and poultry workers in industrialized settings, but few studies have occurred in community settings. In developing countries the potential for zoonotic transmission may be elevated due to inadequate water, sanitation, and hygiene conditions. We collected fecal samples from humans (n= 741), chickens (n=294) and environmental media (n=530) in communities with active small-scale poultry farming operations in rural Ecuador between June 2011-February 2012. Most villages had backyard farms with coops close to households, and one village had a central larger facility. We took advantage of a natural experiment that occurred during the study when the latter village converted to backyard operations between sampling periods. Environmental samples were collected from

domestic (household drinking water and cooking/eating surfaces), outdoor (soil outside households), and coop (soil outside coop and surfaces) locations. We isolated E.coli from all samples and assessed phenotypic resistance to a suite of 12 antibiotics. Isolates were considered multidrug resistant (MDR) if they were resistant to more than 5 antibiotics. We observed high rates of MDR (67-88%) and resistance to fluoroquinolones (enrofloxacin and ciprofloxacin; 50-62%) in coop samples, rates comparable to industrial farming operations. In the village with a collective farming operation, MDR in human samples was significantly elevated during the period when the village had backyard farms (94% of samples MDR) as compared to the when they had a communal facility (28% of samples MDR; p<0.0001). MDR and fluoroquinolone resistant E.coli were also isolated from the domestic environment at higher rates during the time of backyard farming. Small-scale poultry operations are associated with high levels of resistant bacteria in both environmental and human samples in this community setting, especially for farms close to households. These results suggest that it would be safer to promote poultry farming in centralized facilities rather than in backyard operations.

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ANTIBIOTIC RESISTANCE VARIATIONS OF *ENTEROBACTER* SPP. WHICH ISOLATED FROM CYSTITIS OF CHILDREN IN IRAQ

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A total of 57(3.9%) *Enterobacter* spp. were isolated from 1474 urine specimens collected from children (1-7 years) with cysititis, during January to September 2010 at AL-Hakeem hospital in Najaf governorate, Iraq .The results showed that *Enterobacter* spp. were more isolated from females (68.4%) than males (13.6%). The highest month of isolation were recorded during February (6.6%) followed by July (5.8%). The antibiotic resistances of *Enterobacter* spp. isolates showed strongly resistant to Cephaloxin and Cephatoxime (100, 71.4)% and (80, 57.1)% in January and February respectively, while In March the isolates shows a strong resistance to Ceftraiaxone, Cephaloxin and Gentamicin (100%),while in April all isolates show 57.1% resistance to Cephatoxime and 42.8% for Nalidixic Acid while Ceftraiaxone, Nalidixic Acid and Ciprofloxacin were the drug of choice for treatment of *Enterobacter* spp. infections. In July the isolates were resist to Amikacin and Ciprofloxacin in low percentage (0%,11%) respectively. In August and September *Enterobacter* spp. shows a high resistance to Cephaloxin (83.3%,100%) respectively. The antibiotic resistances of *Enterobacter* spp. isolated from cystitis of children were related to seasonal variation in Iraq.

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CAMPYLOBACTER JEJUNI FLUOROQUINOLONE RESISTANCE TESTING BY DETECTION OF THE THR86ILE *GYRA* MUTATION USING REAL-TIME PCR

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Campylobacter jejuni is a major cause of human enteritis, but its treatment has been complicated by the worldwide emergence of resistance to fluroquinolone (FQ) antibiotics, such as ciprofloxacin (CP). FQ resistance is often conferred by Thr86Ile mutation in the *gyrA* gene and may be detected through molecular methods, but few such methods have been evaluated in the developing world. Our objective was to develop and validate a real-time polymerase chain reaction (RT-PCR) Thr86Ile *gyrA* mutation detection assay to detect *C. jejuni* FQ resistance. From 2006 to 2010, *C. jejuni* stool isolates were obtained from three hospitals in Lima, Peru (Hospital Del Niño, Materno Infantil and Emergencias Pediatricas). DNA was extracted by QIAamp DNA Mini Kit. Degenerated primers (773F 5'-CATCTTCCCTAGTCAAGCCT-3' and 773R 5'-AAGATATGGCTCTAGCAAGAC-3') were used to amplify *glyA* fragments and detect a 773-bp PCR product consistent with *C. jejuni*. Isolates confirmed as *C. jejuni* by PCR then had CP susceptibility determined by E-test. Thr86Ile *gyrA* mutation detection was performed by RT-PCR. TaqMan probes (HEX 5'-CCCACATGGAGATACAGCAGTTTATG-3'-BHQ2 and 6-FAM 5'CCCACATGGAGATATAGCAGTTTATG-3'BHQ1), and primers (CjFW gyrA 5'-TGCTGTTATAGGTCGTTA-3' and CjRV *gyrA* 5'-CCTTGTCCTGTAATACTTG-3') were designed by AlleleID-7 software, with the resultant PCR product analysed by Rotor Gene Q. Sensitivity and specificity of the Thr86Ile *gyrA* resistance mutation detection assay was determined using E-test as a gold standard. 189 isolates were confirmed as *C. jejuni*. 74.6% (141/189) of isolates were CP-resistant by E-test. The Thr86Ile *gyrA* mutation was detected in 100% (141/141) of CP-resistant and 0% (0/48) of CP-sensitive isolates. The Thr86Ile *gyrA* mutation detection assay showed a sensitivity and specificity of 100% by comparison to E-test. The RT-PCR *gyrA* Thr86Ile mutation detection assay was highly sensitive and specific in the detection of FQ resistant *C. jejuni*.

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OUTBREAK OF MULTI-DRUG RESISTANT *SALMONELLA* TYPHI, LUSAKA, ZAMBIA, 2011-2012

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Salmonella enterica serovar Typhi causes approximately 22 million typhoid fever infections worldwide each year and outbreaks are increasing across southern Africa as urban population growth overwhelms aging water and sanitation systems. In December 2011, clusters of suspected typhoid cases were identified at two city hospitals in Lusaka, Zambia. From December 2011 - February 2012, 416 suspected cases were reported through hospital-based surveillance. We conducted a retrospective review of laboratory records and a case-control study using hospital-identified cases and matched controls from a highly affected neighborhood reporting an attack rate of 59.1 cases per 100,000 per month to identify risk factors for typhoid fever. A suspected case was defined as a hospitalized person having fever ≥3 days and one or more of the following: abdominal pain, vomiting, diarrhea, constipation, headache, joint pain, muscle pain, malaise, negative malaria parasite test or lack of improvement with antimalarial medication from the affected-neighborhood. Laboratory record review revealed 145 *Salmonella* Typhi isolates recovered from January 2011 - February 2012. Among isolates tested locally for antimicrobial sensitivity, 7/71 (9.9%) were resistant to ciprofloxacin, 9/27 (33.3%) resistant to nalidixic acid, 66/108 (61.1%) resistant to chloramphenicol, and 34/112 (30.4%) resistant to cefotaxime. Of 248 neighborhood households tested, only 20% demonstrated residual chlorination in their drinking water at a level of at least 0.2mg/L. Neighborhood public drinking water sources tested negative for residual chlorine, and one sample was positive for *Escherichia coli*, an indicator of fecal contamination. Recommended control measures included emergency point-of-use water treatment interventions and community education about sanitation and hygiene until long term repairs in water and sanitation systems are made. Antibiotic resistance indicates the need to re-assess treatment recommendations and strengthen laboratory surveillance.

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NOT ALL DEFINED DAILY DOSES ARE EQUIVALENT: EXAMINING THE EFFECTS OF VARIABLE ANTIBIOTIC TREATMENT UPTAKE AND DURATION OF USE ON POPULATION ANTIBIOTIC RESISTANCE

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Antibiotic resistance costs more than $16 billion annually in the United States. Measuring population level antibiotic use is important because it is linked to emergence and prevalence of antibiotic resistance. The WHO advocates a measure called the Defined Daily Dose (DDD) to ascertain the point prevalence of antibiotic treatment in a hospital or community. While this measure takes into account the prevalence of treatment at a given point in time, it does not take into account the rates of treatment uptake and treatment cessation. We use transmission models to assess how variability in starting and stopping antibiotic treatment affects transmission of and competition between sensitive and resistant strains. A given defined daily dose (point prevalence of treatment) can result in a range of possible disease prevalences determined by how quickly people are starting and stopping antibiotic treatment. The faster people are starting and stopping, the lower the transmission strength of sensitive strains. When there are co-circulating antibiotic resistant and sensitive strains, reduced sensitive strain transmission allows resistant strain transmission to increase. Summarizing population level antibiotic use as a defined daily dose provides an incomplete picture of the effect that population level antibiotic use has. Just as the prevalence of disease can be approximated by the incidence of disease multiplied by the duration of disease, so can the prevalence of antibiotic use. Thus combined with the prevalence of treatment, measuring either the rate of uptake or the average duration of treatment would provide a greater understanding of the effects antibiotics have on population level transmission and resistance prevalence.

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ACUTE DIARRHEA IN INDIGENOUS ADULT POPULATION IN NEPAL

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Diarrheal disease is generally accepted as a major cause of childhood morbidity and mortality in developing countries. As population in developing countries have early and repeatedly exposed to enteric pathogens, acute diarrhea in adults is believed to be uncommon or relatively mild and has less public health concerns. Therefore, data on adult diarrhea etiology is scarcely available. We conducted a hospital-based surveillance in an indigenous adult population in Nepal. A stool specimen and demographic and clinical information was collected. Stool culture and identification of enteric bacteria by standard microbiology was conducted in Nepal. Confirmation of isolates and further testing was performed at AFRIMS. Six hundred cases with acute diarrhea and 600 non-diarrhea controls were enrolled. Mean age of cases was 36.5 years, 47% were male, duration of diarrhea before hospital visits was 29 hours and 11% had received prior medication. Ninety four percent of cases reported watery diarrhea and 12% reported blood in stool. Abdominal pain was commonly reported in 80% of cases while only 10% reported fever. The most common organisms detected significantly more frequently in cases than controls were mainly bacteria, *V.cholera* (22% vs 1%), ETEC (10% vs 2%), *Aeromonas* (9% vs 4%) and *Shigella* (8% vs 1%). Enteric viruses, rotavirus, adenovirus or norovirus were also found in 1-4% of cases and rarely in controls. Asymptomatic infections with *Giardia*, Enteroaggregative *E.coli* and Enteropathogenic *E.coli* were also common in Nepalese adults with approximately 9% each. *In vitro* resistance against fluoroquinolone was detected in *Campylobacter* (89%), *Shigella* (15%) and ETEC (9%). In conclusion, bacterial gastroenteritis is common in adults in Nepal suggesting immunity from previous exposure is of short duration or provides incomplete protection. Frequent watery diarrhea and severe abdominal pain were main reasons for adults with acute diarrhea to seek hospital care. Asymptomatic infections with organism frequently colonizing children were also surprisingly common in adults.

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LONG TERM IMMUNE RESPONSES TO THE COLONIZATION FACTOR OF *VIBRIO CHOLERAE* O1, THE TOXIN-COREGULATED PILUS ANTIGEN TCPA, ARE LOW IN MALNOURISHED PATIENTS WITH CHOLERA

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A key adhesion antigen of *Vibrio cholerae* O1 is the toxin co-regulated pilus (TCP) a polymer of TcpA. Following natural cholera, TcpA-specific mucosal and systemic antibodies are induced. A relationship between micronutrient deficiency and development of symptomatic cholera has also been previously observed. We wanted to determine if immune responses including lipopolysaccharide (LPS) and TcpA differ between malnourished and well nourished patients with cholera followed over a year. We included 54 patients with cholera and samples on days 2, 7, 30, 90, 180, 270 and 360 post-onset. Plasma was assayed for antigen specific responses. We assessed nutritional status by determining the "Body mass index" (BMI) and "Mid upper arm circumference" (MUAC) and reference values of the NCHS were used. Patients were grouped as malnourished ('MNour') and well nourished ('WNour').The median age of the study group was 27 years and 35% were females. 89% had severe dehydration in both the groups. The average intake of fluid among 'MNour' group was 4L and 'WNour' group was 8L (p=<0.001) as the same pattern was seen in the ORS intake (p=0.01).Utilizing BMI to stratify patients, IgA responses to TcpA were significantly lower at time points in the 'MNour' group (p=0.006-0.08). The LPS specific IgA antibody responses were also lower in this group (p=0.01-0.04) only from day 180 onwards. When classified by MUAC, IgA responses to TcpA were also lower in the 'MNour' group at day 2 (p=0.05), and from day 90 onwards (p=0.009 to 0.005). The LPS IgA responses were lower among malnourished cases followed up from day 90 to 360 (p=0.002-0.08).No differences were seen between 'MNour' and 'WNour' groups in IgG responses. In summary we show that malnourished patients with cholera have significantly lower IgA immune responses to TcpA and LPS. The relationship of this response with other indicators of protection including the memory B and T cell responses as well as responses in young children need to be evaluated with implications for vaccine intervention in high risk nutritionally deprived populations.

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UNDERNUTRITION BY A REGIONAL BASIC DIET ALTERS SMALL INTESTINAL MORPHOLOGY, BARRIER FUNCTION AND CELL-CELL JUNCTION GENE EXPRESSION IN WEANLING MICE

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Undernutrition is major risk factor for child morbidity and mortality in developing countries, with gut manifestations that include perturbed intestinal epithelial homeostasis, mucosal atrophy, and increased susceptibility to enteric pathogens. The purpose of our study was to evaluate changes in small intestinal morphology, barrier function and

gene expression in mice subjected to malnutrition by a regional basic diet (RBD; 10% protein, 2% fat, 88% carbohydrate). 3 to 4 week old male albino mice were provided an *ad lib* standard chow diet (15% protein, 20% fat, 65% carbohydrate) or the RBD for seven days. On day 7, we assessed weight gain, tail length, and intestinal permeability by the urinary lactulose/mannitol test. Following sacrifice, the ileum was harvested to measure crypt-villous dimensions (height, depth, area), as well as tight junction (claudin-2, occludin, ZO-1) and adherens junction (βcatenin) gene expression by RT-PCR. We found significant decreases in weight (23.8 ± 1.2 g vs. 17.4 ± 0.7 g, p<0.001) and tail length (8.2 ± 0.1 cm vs 7.2 ± 0.1 cm, p<0.01) in mice fed the RBD. Morphometric analysis of ileal villi and crypts revealed reductions in villous area (33940 μm^2 ± 2109 vs 27920 ± 760 μm^2, p<0.01) and crypt depth (160.0 ± 3.4μm vs. 119.5 ± 2.7 μm. p<0.05), but not villus heigh The ratio of urinary lactulose:mannitol was significantly increased in malnourished animals, indicating a greater degree of barrier dysfunction (0.68 ± 0.03 vs. 1.22 ± 0.04 p<0.001). RBD-fed mice showed down-regulation of claudin-2 and ZO-1. The regional basic diet induces failure to thrive in young mice. Our results show important associations between undernutrition, small intestinal morphology, gut barrier function, and alterations in tight junction and adherens junctions gene expression that require further study to elucidate links between nutrition, gut homeostasis, growth, and enteric infections.

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DEVELOPMENT OF A *SHIGELLA* VACCINE BASED ON GENERALIZED MODULES FOR MEMBRANE ANTIGENS

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Shigella is one of the most frequent causes of diarrhea in young children and infants in developing countries. In natural infection the immune response to the O antigen is protective but serotype-specific and *Shigella* comprises 50 different serotypes. No vaccine is available. Novartis Vaccines Institute for Global Health's not-for-profit mission is to develop effective and affordable vaccines for impoverished communities. Gram-negative bacteria naturally shed particles that consist of outer membrane lipids and outer membrane proteins in their native orientation and of soluble periplasmic components. These particles have been proposed for use as vaccines but the yield has been problematic. We developed a high yielding production process of genetically derived outer membrane particles we named Generalized Modules for Membrane Antigens (GMMA, also known as outer membrane vesicles). Yields of approximately 100 milligrams of membrane-associated proteins per liter were obtained from high density cultures of genetically modified *S. sonnei* in a 5 L fermenter supporting the feasibility of scaling up this approach as an affordable manufacturing process. Proteomic analysis of the purified particles showed the preparation to primarily contain predicted outer membrane and periplasmic proteins. Furthermore, we demonstrated the feasibility of using this process with other genetic manipulations, e.g. to reduce LPS endotoxicity and to modify immunogenicity by removing the immunodominant O antigen. GMMA were shown to confer protection against *Shigella* using the murine model of pulmonary infection. Intranasal immunization with GMMA with and without O antigen derived from *S. flexneri* 2a resulted in statistically significant survival after homologous challenge compared to placebo. Importantly, GMMA without O antigen derived from *S. sonnei* also protected against challenge with *S. flexneri* 2a, demonstrating the potential for heterologous protection. In conclusion, this work provides the basis for a large scale manufacturing process of GMMA for production of vaccines from Gram-negative bacteria and for the development of GMMA as vaccine candidate against *Shigella*.

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CHOLERA OUTBREAK IN TOFA AND SAMAWA WARDS - ZAMFARA STATE, NIGERIA; 2011

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Cholera remains a major public health problem in Nigeria usually occurring as large scale outbreaks leading to high morbidity and mortality. Between June-July, 2011; we investigated suspected cholera outbreak in Tofa and Samawa wards in Bungudu Local Government of Zamfara State, northern Nigeria to confirm the outbreak and implement targeted interventions. We conducted cross sectional study. A suspected cholera case was defined as any resident of Tofa or Samawa ward with at least three episodes of acute watery diarrhea with or without vomiting between 30th May and 4th July, 2011. We reviewed hospital records and used case-based line listing to collect patients' data. Environmental assessment was conducted. We collected and analyzed 5 stool and 2 water samples using Thiosulfate Citrate Bile Salts Sucrose and Mac Conkey agars. Data were analyzed using Epi info and Microsoft Excel. Altogether, 111 cases were recorded (attack rate: 0.59%) with 5 deaths (Case fatality rate: 4.5%). Females accounted for 50.5% (56) and males 49.5% (55) of cases (p= 0.9), age range was 4 months to 65 years, median age was 5 years. About half of cases (47.7%) were children between 0-4 years (p = 0.01), age-specific attack rate for 0-4 years age group = 1.06%. All (100%) stool specimens yielded V. cholerae 01, serotype Ogawa. Environmental assessment revealed unsanitary conditions and inadequate and unsafe water supply. Water samples tested negative for V. cholerae but yielded growths of *E. coli*. Bungudu LGA had confirmed cholera outbreak. We strengthened case management and conducted health education focusing on personal hygiene and environmental sanitation. Advocacy visit was paid to local authorities to intensify health education and provide adequate and potable water to affected communities.

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GLOBAL AND REGIONAL TRENDS IN CONTINUED FEEDING OF CHILDREN LESS THAN FIVE YEARS OLD WITH DIARRHEA: ANALYSIS OF DEMOGRAPHIC AND HEALTH SURVEY DATA FROM 1993 TO 2010

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Continued feeding is a critical component of successful home management of diarrhea in children, and has been associated with decreased mortality and incidence of diarrhea. Repeated episodes of diarrhea are associated with malnutrition in children, and feeding a child during and after diarrhea episodes is crucial in preventing adverse nutritional effects. Understanding region-specific trends in continued feeding practices for children with diarrhea would allow appropriate direction of efforts to prevent deaths in the regions where diarrhea-associated mortality is greatest. We examined global and regional trends in continued feeding during diarrhea episodes among children less than 5 years old using Demographic and Health Survey (DHS) data from DH conducted between 1993 and 2010 in Asia, Latin America, and Sub-Saharan Africa. Continued feeding prevalence estimates were weighted by each country's population of children less than 5 years old for each year. We performed linear regression to estimate the change per year (β in prevalence of continued feeding during diarrhea globally and by region. The population of children less than 5 years of age represented by the 89 DHS in this analysis was 589 million. Prevalence of continued feeding for each region as estimated from recent DHS surveys (2008- 2010) ranged from 33.3-49.8% in Latin America, 33.7-61.1% in Sub -Saharan Africa,

and 35.9-71.1 % in Asia. Between 1993 and 2010, globally, continued feeding for children with diarrhea appears to be unchanged (β=0.15, p=0.82). Likewise, no significant improvements in continued feeding were observed in Latin America (β= -0.10, p=0.78) or Sub-Saharan Africa (β= -0.15, p=0.27). Prevalence of continued feeding in Asia appears to be increasing significantly at 0.25% increase rate per year (p=0.05). Sub-Saharan Africa has the highest diarrhea-associated mortality of any region in the world but has seen no improvements in continued feeding for children with diarrhea since 1993.These findings indicate the need for quantitative and qualitative research to understand barriers to continued feeding on the part of caregivers, and renewed efforts to promote continued feeding as a core component of diarrhea case management in settings where the burden of diarrhea is high.

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TRENDS IN ANTIBIOTIC RESISTANCE AND DIARRHEAL DISEASE EPIDEMIOLOGY IN A MILITARY POPULATION IN THE PERUVIAN AMAZON, 2003-2011

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In Peru, where antibiotic use is unregulated, the effective treatment of diarrheal disease is often complicated by the development of antibiotic resistant organisms. We aimed to investigate the trends in diarrheal disease etiology and antibiotic resistance in a military population in the Peruvian Amazon in order to guide diarrhea treatment. From 2003 to 2011, diarrheal disease surveillance was conducted among personnel at the Vargas-Guerra Army Base in Iquitos, Peru. All individuals experiencing diarrhea were requested to present to the army health post where a stool sample was taken for culture. Diarrheagenic bacteria were isolated from 34.5% of the 638 cases. From the 215 samples in which a single bacterial pathogen was isolated, *Shigella flexneri*, *Enterotoxigenic E. coli* (ETEC) and *Enteroinvasive E. coli* (EIEC), were the most common pathogens and were identified in 47.0%, 30.2%, and 5.6% of samples, respectively. There were no trends in the prevalence of *Shigella flexneri* or EIEC over the study period, however, the prevalence of ETEC decreased significantly (Odds Ratio= 0.86, 95% CI=0.75, 0.97; p=0.02). Of 101 isolates of *Shigella flexneri* cultured, 95.0% demonstrated resistance to tetracycline, 89.1% to chloramphenicol, 84.2% to ampicillin, and 80.2% to cotrimoxazole. Resistance of *Shigella flexneri* to ciprofloxacin and azithromycin remained low (0% and 8.9%, respectively). There were no significant trends in resistance to any other antibiotics over time. These data demonstrate a high prevalence of *Shigella flexneri* and diarrheagenic *E. coli* among diarrhea cases in a military population in the Peruvian Amazon. Although antibiotic resistance to penicillins and sulfa antibiotics remains high in this population, more appropriate or less frequent use of certain antibiotics may have led to decreasing resistance.

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CLUSTER OF GUILLAIN-BARRÉ SYNDROME DUE TO A WATERBORNE OUTBREAK OF *CAMPYLOBACTER JEJUNI* INFECTION -- SAN LUIS RIO COLORADO, SONORA, MEXICO AND YUMA, ARIZONA, UNITED STATES, 2011

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From May 31-June 16, 2011, a cluster of 15 suspected cases of Guillain-Barré Syndrome (GBS) which sometimes follows *Campylobacter jejuni* infection, was reported in San Luis Rio Colorado (SLRC), Sonora, Mexico and Yuma County (YC), Arizona. Epidemiological teams from Mexico and the United States conducted a binational outbreak investigation to confirm this cluster and determine the etiology. We performed additional case-finding and classified GBS cases through interviews and medical record review. To investigate exposures, we reviewed disease surveillance data, performed *C. jejuni* stool culture, conducted a case-control study examining food and water exposures of cases with GBS or *C. jejuni* infection, and performed an environmental assessment of water systems. From May 4-July 21, 2011, 16 SLRC residents and 8 YC residents developed GBS, far exceeding the expected number of cases. Twenty-one GBS patients (81%) reported antecedent diarrhea. Approximately two weeks before this cluster, weekly YC *C. jejuni* reports doubled compared with the 3 previous years. Though *C. jejuni* diagnostics were limited, 2 GBS patients had stool cultures yielding *C. jejuni* and 4 others had positive serologic or stool antigen tests. In the case-control study, all 7 GBS case-patients from YC traveled to SLRC during the exposure period versus 37% of 19 matched controls (mOR: 10.2; CI: 1.4-inf.). Few case-patients or controls (<20%) drank tap water, but >95% reported exposure through other routes. Case-patients consumed more washable, uncooked produce items than controls (Median: 7 vs. 5; P = 0.04). The SLRC municipal water system had a history of inadequate chlorination and pipe disruptions. Inadequately disinfected tap water contaminated with *C. jejuni* was the likely source of this first mainland North American outbreak of GBS. Improved water treatment practices were implemented and the institution of new epidemiological surveillance strategies in SLRC since this investigation will improve early detection of diarrheal outbreaks and facilitate public health actions.

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IDENTIFICATION AND CONFIRMATION THROUGH MULTIPLEX PCR OF THE SPECIES OF *ARCOBACTER* IN ISOLATES FROM HUMAN AND ANIMAL FECAL SAMPLES IN LIMA, PERU

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A multiplex PCR was used to confirm the identity of isolates that are phenotypically suggestive of *Arcobacter* from human and animal stool samples in Lima, Peru. We evaluated 57 bacterial isolates from human fecal samples (3), pigs (52), lion (1) and rabbit (1), with the following phenotypic characteristics suggestive of *Arcobacter*: gram-negative rods, curve-shaped C or S, mobile, aerobic and microaerophilic, circular colonies 1 to 2mm in diameter at 18-24 hours of incubation in microaerophilic conditions on blood agar, non-hemolytic and lactose negative colonies on MacConkey agar, oxidase and catalase positive. The study of genotype was performed by multiplex PCR, as reported previously, using primers targeting the 16S and 23S rRNA genes for the detection of three species*: A. butzleri, A. cryaerophilus, A. skirrowi*, with a molecular weight of 401-bp, 257-bp, 641-bp, respectively. It was confirmed molecularly that Arcobacter was in 87.7% (50/57) of the isolates studied, 90% (45/50) of which corresponded to *A. butzler*, 8.0% (4/50) to *A. cryaerophilus* and 2% (2/50) *to A. skirrowi*. A proportion of 12.3% were negative with the primers used. Of the three human samples, two isolates corresponded to *A. butzleri* and one to *A. cryaerophilus*. The rabbit and lion isolates were *A. butzler*i. Of the pig isolates, 91.1% (41/45) *A. butzler*, 6.7% (3/45) were *A. cryaerophilus*, and 2.2% (1/45) *A. skirrowi*. The presence of *A. butzler*, *A. cryaerophilus* and *A. skirrowi* were confirmed in 87.7% of the total isolates of human, pig, rabbit and lion fecal samples. The remaining 12.3% of the isolates are likely composed of other species of *Arcobacter*.

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RISK PRACTICES REGARDING ANIMAL AND HUMAN ANTHRAX IN BANGLADESH: AN EXPLORATORY STUDY

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From August 2009 to October 2010, there were 14 outbreaks of anthrax in Bangladesh that included 140 animals and 273 human cases. A collaborative team of icddr,b and IEDCR undertook a qualitative investigation to explore livestock rearing practices, the handling of sick and dead animals and the anthrax vaccination programme among outbreak-affected communities as potential contributors to the animal anthrax outbreaks. We conducted a qualitative study in 5 anthrax outbreak-affected villages in 2009 and 2010. To explore butchering sick animals and carcass disposal practices, we conducted in-depth interviews, observation and group discussions with the owners of sick cattle and people who participated in butchering activity. We used key-informant interviews with local government livestock officers to explore the supply and delivery of anthrax animal vaccine. Farmers in these areas raised cattle, goats and/or sheep in their courtyard, fed them dry and green rice straw, green grass gathered from the pastures, rice husk, wheat bran, and oil cake made locally from mustard or sesame seeds. They also grazed the livestock in pastures. Cattle represent a significant financial investment, so when sick cattle were on the verge of death, cattle owners and their neighbors and friends often rapidly slaughtered the cow near or inside the cowshed. The farmers reported discarding the carcasses and butchering waste in nearby ditches, flood waters or open fields. A few carcasses were buried near the owners' house, but often these were dug up by scavenging dogs and foxes. Skinners removed the hides from discarded carcasses and sold them in the local market. Government livestock officers reported that cattle in these outbreak communities did not receive routine anthrax vaccination due to shortage of vaccine and manpower. In conclusion, the slaughter of anthrax infected animals and disposal of butchering waste and carcasses in environments where ruminants live and graze, combined with limited vaccination, provided a context that permitted repeated anthrax outbreaks. Because of strong financial incentives, slaughtering and discarding moribund animals will likely continue. Surveillance for earlier detection of anthrax outbreaks, and better vaccination coverage for at-risk animal population may reduce animal and subsequent human infection.

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EFFECT OF GALANTAMINE ON TULAREMIA PATHOGENESIS IN A BALB/C MOUSE MODEL

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Galantamine is an inhibitor of acetylcholinesterase able to interact with nicotinic acetylcholine receptors as well. Owing to the significant role of cholinergic anti-inflammatory pathways in neuro-immunomodulation, we aimed our effort to examine the effect of galantamine on tularemia-infected BALB/c mice. Animals were infected with *Francisella tularensis* LVS and treated with galantamine in a total amount 0.1 mg/kg of body weight. We examined total mortality, interleukin 6 (IL-6) and interferon gamma (IFN-β levels using enzyme-linked immunosorbent assays using plasma samples. Beside the cytokines assay, the following biochemical markers: inorganic phosphate, uric acid, lactate dehydrogenase, gamma glutamyltransferase, creatinine phosphokinase and amylase were assayed using an automated device. The two opposing processes were proven in the laboratory animals in course of galantamine after tularemia infection: up regulation of IFN-βand down regulation of IL-6. In compliance with expectations, tularemia infection resulted in damage of kidneys, as hyperphosphataemia and hyperuricaemia were proven in the infected animals. Surprisingly, galantamine resulted in calming down of the nephropathy. Markers of kidney dysfunction were modulated as well. The most surprising parameters was alteration of mortality in course of tularemia. As mentioned above, galantamine can significantly influence the immune response. Owing to the results, we infer implication of the cholinergic anti-inflammatory pathway.

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CHARACTERIZATION OF A MURINE MODEL FOR HUMAN SCRUB TYPHUS

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There are over one million scrub typhus cases annually with one billion people at risk of being infected, illustrating the importance of scrub typhus in global health. *Orientia tsutsugamushi*, the etiologic agent of scrub typhus, is a rickettsia transmitted by the parasitic larval stage of trombiculid mites. *O. tsutsugamushi* Karp strain (OTK) has been used extensively in mouse studies using various inoculation strategies with little success in inducing disease progression similar to that seen in human cases, but inducing inoculation route-specific pathology. Intravenous injection of spotted fever and typhus group rickettsiae result in disseminated endothelial infection that causes similar pathology to the human diseases. The objective of this project was to develop a disease model that demonstrates pathology and target cells similar to those of severe human disease. Development of this model will allow for investigation of the immunological mechanisms that mediate protective immunity in scrub typhus infections. This study reports an intravenous infection model under development in our laboratory. C57BL/6 (B6) mice were determined to be susceptible to intravenous challenge by OTK with overt signs of illness with a dose dependent time of onset. Lethal infection occurred after intravenous inoculation of 1.25×10^6 focus forming units (FFU) of OTK with an LD_{50} of approximately 1.25×10^5 FFU. Signs of illness began on day 7 with death occurring ~3-5 days later. Immunohistochemical staining for OTK antigens demonstrated extensive endothelial infection, most notably in the brain and lungs. The histopathology revealed cerebral perivascular lymphohistiocytic infiltrates, focal hemorrhage, meningoencephalitis, and interstitial pneumonia. Intravenous inoculation of *Orientia tsutsugamushi* Karp strain resulted in a disseminated endothelial infection with pathology in B6 mice resembling that of human scrub typhus. A dose dependent severity was also established, providing an avenue to elucidate determinants of severity in scrub typhus infections.

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INVASIVE METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* INFECTIONS AMONG CHILDREN IN BAMAKO, MALI

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Infections due to methicillin-resistant *Staphylococcus aureus* (MRSA) are increasing worldwide. The clinical spectrum of the disease ranges from nasal colonization to superficial and invasive infections. There is a paucity of data about MRSA disease in the pediatric Malian population Hospitalized children aged 0-15 years and ambulatory patients aged 0-35 months evaluated at Hopital Gabriel Toure, the main pediatric hospital in Bamako, with fever ≥39°C or syndrome compatible with invasive bacterial infection were invited to participate in a study in which blood

and relevant body fluid; e.g. cerebrospinal fluid (CSF)) were cultured to identify *S. aureus*; Hospitalized children were followed until discharge and outpatients were contacted if the culture result was positive. From January 2007 to December 2011, 10750 hospitalized children were enrolled, 5600 ambulatory patients were enrolled. MRSA was isolated from 147 inpatients (1.3%) and 23 outpatients (0.4%). Among inpatients, 70 cases (47.6%) occurred in 0-to- 11 month old infants, 19 (12.9%) in 1-t0-4 years old and 58 (39.4%) in 5-to- 15 year olds. 110 isolates (74.8%) were blood only, 15(10.2%) from under skin fluid, 13 from pleural fluid(8.8%), 4 from muscular fluid (2.7%), 3 from joint fluid(2%) and 2 from cerebral spinal fluid(1.3%). 46(31.2%) inpatients died. Among outpatients, 15 (65.2%) cases occurred in infants and 8(34.7%) in 12-to- 35 month olds. 18 isolates (78.2%) were blood only, 4 from muscular fluid (17.3%) and 1 from under skin fluid (4.3%). Follow-up of ambulatory revealed that 15 children had improved, 4 were had not and 4 could not be located. In conclusion, MRSA is common and frequently fatal among children in Mali.

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SALMONELLA MENINGITIS AMONG CHILDREN HOSPITALIZED IN BAMAKO, MALI

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Bacterial meningitis are medical and therapeutic emergency. Three microorganisms (*Salmonella pneumoniae, Neisseria meningitidis*, Hib) are responsible of case majority. Now a day other micro-organisms are observed like *Salmonella*. That why we are doing this study to observe *Salmonella's* part among meningitis epidemiologic. Hospitalized children aged 0-15 years evaluated at Hopital Gabriel Toure, the main pediatric hospital in Bamako, with fever ≥39°C or syndrome compatible with invasive bacterial infection were invited to participate in a study in which blood and relevant body fluid (e.g. Cerebrospinal fluid(CSF)) were cultured to identify *Salmonella*; Hospitalized children were followed until discharged. From January 2008 to December 2011, 7403 hospitalized children were enrolled; *Salmonella* was isolated from 22 inpatients (5.4%). Among patients, 3 cases (13.6%) occurred in 0-to- 1 month old infants, 12 (54.5%) in 1-t0-23 months old, 5 (22.7%) in 24-to- 59 months old and 2(9,1%) in olds > 59 months. *Salmonella* D was the most strain isolated (54,5%) follow by Salmonella spp (22,7%), *Salmonella* B (18,2%),*Salmonella* E or G (4,5%),6(27.3%) patients died. *Salmonella* was most sensible to the ciprofloxacin (100%) and ceftriaxone (95, 5%). The majority of patients (36, 4%) were hospitalized during over ten days. In conclusion, meningitis with salmonellae is common particularly among children from 1 to 23 months old and frequently deadly among children in Mali.

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A COLLABORATIVE RESEARCH AND ADVOCACY EFFORT FOR TYPHOID FEVER CONTROL IN NEPAL: THE PILOT INTRODUCTION VACCINATION PROGRAM

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Typhoid fever continues to be a public health problem in many developing countries. Nepal is one of the highest typhoid incidence countries and infamously referred as "*Enteric fever capital of the world*". Typhoid Vi polysaccharide vaccine has been in market by multiple producers now after the first trial was published in lancet in 1987 by Acharya et al. Typhoid Vi polysaccharide vaccine is known to be safe and moderately efficacious. Few countries (China, Cuba, Vietnam, and the State of Delhi in India) have introduced typhoid vaccine into a public health program. The Vi-based Vaccines for Asia (VIVA) Initiative initiated a joint collaborative effort with the government and non-government partners to introduce typhoid Vi polysaccharide vaccine as part of their public health program. Prior to the start of the program initial meetings were conducted with the health officials to have their opinion on the use of typhoid vaccine in Nepal. Later through qualitative research perceptions of target population and major stakeholders were assessed to guide the formation of a communication strategy to increase awareness about the disease, control measures and importance of vaccination. Regular meetings with health officials, national and international non-for profit organizations, and district education office were conducted in parallel to project field activities. District Lalitpur was chosen for the pilot program of the program. Typhoid fever control has made it's place in the list of government's list of high priority diseases. It has been recommended by the national committee on immunization practices that typhoid vaccines should be used to control the disease. Typhoid vaccination has also be included in the five year health plan of the government. The formal and informal meetings played an important role in the advocacy efforts of creating awareness for typhoid control in Nepal. The government of Nepal has always been committed to control of disease through vaccination, however, the efforts and results from studies conducted under VIVA initiative provided evidence for policy decisions.

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EPIDEMIOLOGICAL AND MICROBIOLOGICAL DIAGNOSIS OF BOVINE TUBERCULOSIS IN SLAUGHTERHOUSES, BURKINA FASO

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Bovine tuberculosis is still unknown in Burkina Faso despite the importance of livestock. The disease is widely suspected at slaughterhouses, but not supplemented by laboratory tests in the country. This study aimed to investigate tuberculosis in bovine carcasses through lesions in lymph nodes or organs at routine inspection, and to identify the causal strains. A prospective study conducted in two slaughterhouses located at Ouagadougou and Bobo-Dioulasso from May-October 2011. A structured questionnaire administered to the owners of suspected carcasses at routine inspection to collect epidemiological data. Sample of lymph node or organ injured collected from suspected animals, and submitted to microscopic examination after Ziehl Neelsen coloration and to bacterial culture using Lowenstein-Jensen ordinary and enriched mediums. From a total of 1499 carcasses examined, 101 showed suspicious lesions of tuberculosis representing a prevalence of 6.07%. The distribution of injuries shows frequently a violation of respiratory lymph nodes with a rate of 92.08%. The isolation and identification of isolates have confirmed 48 positive cultures at a rate of 51.54%. Among these, 47.92% are represented by *Mycobacterium bovis*, 6.25% by *M. africanum*, 4.17% by *M. tuberculosis* and 41.66% by non tuberculosis mycobacteria strain. In conclusion, bovine tuberculosis circulates in Burkina Faso and *M. bovis* is one of potential causative strains of this disease. Other strains of *Mycobacterium* such as M. *africanum* and *M. tuberculosis* were also identified.

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DEVELOPMENT AND VALIDATION OF A QUANTITATIVE PCR (TAQMAN) ASSAY FOR DETECTION AND EARLY DIAGNOSIS OF *LEPTOSPIROSIS INTERROGANS* USING THE JOINT BIOLOGICAL AGENT IDENTIFICATION AND DIAGNOSTIC SYSTEM (JBAIDS)

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Leptospirosis is a potentially serious disease often mistaken for other acute febrile illnesses because of its nonspecific presentation. Current gold standard methods for leptospirosis diagnosis, the microscopic agglutination test (MAT) and an ELISA assay, have several limitations for early diagnosis because of technical complexity and low sensitivity. Diagnostic tests that generate results quickly, cheaply, and definitively are needed. We are addressing this clinical diagnostic need by developing a real-time PCR TaqMan assay using a freeze-dried real time PCR on the Joint Biological Agent Identification and Diagnostic System (JBAIDS) platform for the rapid detection of *Leptospira* bacteria in field samples. Our assay uses primers and probes targeted to highly conserved leptospiral outer membrane proteins (OMPs) on the lipl32 gene. We assessed a 132 bp target for detection of *Leptospira* human pathogenic species using dual-fluorogenic hydrolysis probes. Quantification, accuracy and precision of the assay were determined through serial dilutions of *L. interrogans* serovar Autumnalis genomic DNA, representing a strain of reemerging infectious disease and a major causative agent in Northeast Thailand. We determined assay sensitivity using tenfold serial dilutions in duplicate, ranging from 10 ng to 1 fg. The assay consistently successfully detected as low as 10 fg or 2 genomic (ge) equivalents. The limit of detection was very low at 100 fg or 20 ge. We tested a diverse panel of pathogenic and non-pathogenic *Leptospira* reference strains, genetic near neighbors, and human DNA. Our results suggest this method is a highly sensitive and specific diagnostic tool for identifying pathogenic leptospirosis. Standard diagnostic tests using the MAT and ELISA methods were used to classify serum samples collected in 1999-2002 from patients admitted to a hospital in Kanchanaburi province, Thailand, with a febrile illness and clinical suspicion of leptospirosis. Using the results of these assays, 17.5% of patients were positive for leptospirosis. We are currently evaluating the clinical utility of our JBAIDS assay, compared to the sensitivity and specificity of the standard MAT and ELISA methods, for detecting pathogenic species of Leptospira in these and other clinical samples from Thailand.

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CYSTIC ECHINOCOCCOSIS IN SUDAN AND SOUTH SUDAN: PAST AND FUTURE OF AN IMPORTANT NEGLECTED DISEASE

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Cystic echinococcosis (CE) is a zoonotic disease affecting mainly various species of livestock and humans. It is caused by metacestodes of dog tapeworms of the *Echinococcus granulosus* complex. The metacestodes usually form fluid filled cysts ('hydatids') located in liver, lungs and other organs. CE is distributed world wide, acquiring public health or economic significance in areas where extensive livestock production provides suitable conditions for the cyclic transmission between dogs and other animals which can serve as intermediate hosts. It is considered an emerging disease in many parts of the world, in some regions re-emerging after initially successful control. The global burden of CE is estimated at >1,000,000 DALYs (disability adjusted life years) lost, which gives CE a greater impact than onchocercosis, Dengue fever and Chagas disease, and approaches the burden caused by African trypanosomosis and schistosomosis. CE has been reported from the majority of countries in subsaharan Africa. However, as it is typically a disease affecting pastoral communities which often live in remote areas. reliable data on prevalence of CE in humans or animals are only known from few regions. In livestock, CE seems to be widespread and frequent especially in eastern and southern Africa. In contrast, high-prevalence regions of human CE are focally distributed in some African countries including Southern Sudan. In Sudan, all epidemiological conditions for autochthonous transmission of cystic echinococcosis (CE) are given: In rural areas there are large numbers of dogs in and around villages, and infection can occur with offal from slaughterhouses or during unsupervised home slaughtering. the disease seems to occur sporadically in human in a large part of the country. Nevertheless, even if the parasite may have lower infectivity to humans, the infection can occasionally get established and progress to clinical CE. This study aims at highlighting of the course of research on CE in different animals and humans in Sudan since the disease was first reported in 1908. Recent data about the genetic identification of the parasite in the country in both humans and animals and its importance for future control programs is discussed.

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A CASE OF GIANT SPLENIC HYDATIDOSIS

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Hydatidosis is a parasitic disease caused, in most cases, by a parasite called *Echinococcus granulosus*, this is an endemic disease present in all the continents of the world. In Peru, a South American country, the prevalence of this disease commonly present in the central andes reaches about 9%. Frequently people acquire the disease by the breeding of dogs and the habit of feeding these animals with guts of cattles and sheeps that are infected with the parasite, the Echinococcus can live in the intestines and feces of a contaminated dog, who contaminates the water and food, the eggs from this parasite are accidentally ingested by humans, as a result people acquires the disease. The hydatid cyst in humans is located in the liver at a rate of 70% and in the lung about 30% of the time, the reported prevalence of hydatid cyst of the spleen ranges between 0.9% to 8% of the cases, and as a primary presentation less than 2%, making it quite rare. We present here a case of primary hydatid cyst located in the spleen of a 75 years old Peruvian woman, the diagnosis was made by ultrasonography and computed tomography scan which showed a giant hydatid cystic mass located under the spleen in the left hypochondrium measuring 165 x 130 mm of diameter, the other abdominal and thoracic organs were normal, the western blot test was positive for hydatid disease. The patient was operated laparoscopically without any complications, postoperative evolution was favorable, pharmacological treatment was performed with albendazole 400 mg every 12 hours, in 3 cycles with one-week break between them.

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PHYSIOLOGICAL AND PATHOLOGICAL LIVER PROFILE CHANGES AFTER *IN VITRO* AND *IN VIVO* EVALUATION OF THE THERAPEUTIC EFFECTS OF NEW HETERO-CYCLIC ORGANIC COMPOUNDS AS THIAZOLE, TRIAZOLE, PYRIDAZINE AND PYRIMIDINE, IN COMBINATION WITH ALBENDAZOLE, AGAINST *ECHINOCOCCUS* METACESTODES

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The metacestode (larval) stage of the tapeworm *Echinococcus multilocularis* causes alveolar echinococcosis (AE), a mainly hepatic disease characterized by continuous asexual proliferation of metacestodes by exogenous budding resulting in tumor-like infiltrative growth of the parasite lesion leading to physiological and pathological deterioration of the compressed hepatic cells function. Current chemotherapeutical treatment of AE relies on the use of benzimidazole (albendazole, mebendazole), but these drugs act as parasitostatic rather than parasiticidal, and in case of side effects such as liver toxicity, patients are left without valuable alternatives. New hetero-cyclic compounds as thiazole, triazole, pyridazine and pyrimidine with a documented anti-inflammatory, anti-bacterial and anti-fungal effects have been investigated. All compounds have been evaluated *in vitro* for their anthelmintic activities against *E. multilocularis* metacestodes. Some compounds showed a great nematicidal activity (LC 100) ranging between 0.0005 and 0.002 ug/ml. First the compounds *in vitro* treatment downscaled the transcription of the 14-3-3-pro-tumorogenic zeta-isoform in *E. multilocularis*. Second, scanning and transmission electron microscopy showed that the germinal layer of *E. multilocularis* was dramatically damaged following treatment confirmed by using alkaline phophatase as a marker for metacestode damage. This therapeutic effect of the new synthetic hetero-cyclic organic compounds was dose dependent. Similar results were obtained with *E. granulosus* metacestode. Bioassays were performed in *E. multilocularis* experimentally infected mice treated with the new hetero-cyclic compounds alone, albendazole alone and combination of both. Best results were achieved with a comination of the new synthetic hetero-cyclic compounds and albedazole. This study has shown that hetero-cylic organic compounds as thiazole, triazole, pyridazine and pyrimidine derivatives are promising candidates for the development of new anthelmintic agents with a rapid regeneration and hepatic physiological and pathological functional reformation of a damaged compressed liver tissue caused by the tumor-like infiltrative growth of the parasite hepatic lesions.

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CATHETERIZATION OF GIANT ECHINOCOCCAL CYSTS OF THE LIVER: SINGLE CENTER EXPERIENCE

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Giant echinococcal cysts have a diameter 10 cm, and are usually treated with a surgical approach even when they are CE1 or CE3a because standard PAIR procedures cannot achieve complete solidification . Following the pioneering work by Men *et al*., who treated giant CE1 and CE3a cysts by a modified catheterization technique and left the catheter inside the cavity until daily drainage was less than 10 ml, we used percutaneous catheterization in 6 patients with giant echinococcal cysts of the liver. A 6 or 8 F pig-tail catheter was inserted into the cyst under sonographic guidance in the presence of an anesthesiologist. The trans-hepatic approach was chosen in five cases and the trans-costal access in one case. The Men's protocol was simplified by avoiding the injection of any scolecidal and sclerosing agents as all patients were already receiving albendazole. The mean hospital stay was 6 days (range 3-18). All patients were discharged with the catheter still inserted but disconnected from the collecting bag, and returned to our clinic on average every five days (range 2-15) to drain any fluid that may have re-accumulated. The catheter was left inside the cyst until the output decreased to less than 20 ml and the cystic wall appeared permanently collapsed. For all patients the mean catheterization time was 30 days (range 10-60). All patients except for two in which the catheter slid out accidentally during the hospital stay had a solid (CE4) cyst after a mean follow-up of 8 months (range 0-26) . No bacterial infections were observed. Although these are preliminary results, continuous catheterization seems to be a promising alternative to surgery for the treatment of giant echinococcal cysts of the liver. Studies on larger series are needed.

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CYSTIC ECHINOCOCOSIS CLUSTERS AT HOUSEHOLD LEVEL

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Human cystic echinoccocosis is endemic in many regions including some industrialized countries. Its epidemiology at the community level has been widely studied using either imagenological or serological tools. A known and obvious risk factors is contact with dogs, which in turn get infected because of risk practices (home slaughtering, releasing infected offal into the environment). Whether people living in the same household with a CE case have a higher risk of also having the disease has been suggested but not yet demonstrated. This study aimed to determine if household members or people who live close to a case have a higher probability to have CE. Cluster analysis at household level was preformed with data from a rural endemic community in the Peruvian highlands. The community (330 households, 1380 inhabitants) was divided in 6 sectors, and all inhabitants were invited to participate in the survey. The number of members per house varied from 1 to 8 members. One hundred and ten households participated in the survey, with a coverage of 35% of members per house. Fifteen households have at least one infected member (15/110, 14%) and 3 of them had more than one member. Three sectors had more than one household with infected members. The presence of a CE case is associated to an increased likelihood of having another household member infected

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LOOKING FOR THE HABITUAL INTERMEDIARY HOST OF *ECHINOCOCCUS GRANULOSUS* G6 STRAIN IN PERU

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Cystic echinococosis is a zoonotic disease widely distributed around the world, with sustained endemicity in farming countries like Peru. In the last years, with the development of molecular tools, the identification of *Echinococcus granulosus* strains has acquired importance. Some studies refer that strain could influence disease characteristics (e.g. clinical presentation, organ infected, response to treatment, etc). In Peru, a previous study found that human cases were infected by G1 and G6 strains. Since the reservoir of the G6 strain remains undetermined in Peru, the aim of the present study was to determine if goats are the habitual intermediary G6 host. We collected cysts from goats slaughtered in formal and informal abattoirs located in two coastal cities receiving animals from surrounding endemic areas. Information about involved organ, size of cysts, macroscopical evaluation of cysts stage and involution, as well as goat age, sex and geographical origin were recorded. Isolates were identified by DNA sequencing of the mitochondrial cytochrome c oxidase subunit 1 gene. A total of 71 cysts were collected. Initial analysis in 22 cysts found G6 in 68.18% (15/22) and G1 in 31.82% (7/22) of them. Most cysts (20/22, 90.91%) were located in the lungs and only 2 cysts were located in the liver. The above preliminary findings suggest that goats may as reservoirs for G6 in Peruvian endemic areas. Control strategies may be adapted to these hosts to improve the control of parasite transmission

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A 15-YEAR EXPERIENCE WITH HYDATID DISEASE IN A NON-ENDEMIC REGION

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Hydatid disease is a common zoonosis caused by the larval cysts of *Echinococcus granulosus*. The disease is endemic in parts of the world where there is intimate contact between man and the definitive and intermediate hosts, usually sheep and dogs, but there are no large series from non-endemic regions of the world. We report 37 cases over a 15 year period from a Tropical Medicine Clinic in the Bronx. Of these, 23(62%) were male with a mean age of 44±13 years and mean time from immigration 31±14 yrs. All were immigrants, with the majority from Albania, Yugoslavia, Italy, Macedonia,Yemen, Peru and Tibet. Cysts were located in: liver 31(84%), lung 4(11%), bone 3(8%), brain1 (3%). Of the cysts located in the liver the staging was CE1 2(7%),CE213 (42%), CE3b 4(14%), CE4 7(23%), CE5 (3%), 2 unknown and they were followed for a median of 1.4 yrs (6mo.-16yrs). Two large CE2 cysts treated medically elsewhere presented later with devastating consequences. Complications were seen in 10 (32%) of liver cysts with 3 cystobiliary fistulas, 1 abscess, 5 IVC involvement; one ruptured into the intrahepatic IVC and developed pulmonary embolism and hypertension. Two patients ruptured into the peritoneum spontaneously and one ruptured into the liver after trauma. Three liver cysts penetrated the diaphragm. Of those that underwent surgery, 30% recurred. The 3 patients with bone disease presented with neurologic symptoms or pathologic fracture and despite surgery and medical therapy have not been cured and have chronic, debilitating disease at five years. Two pulmonary cases were treated medically; one ruptured into the mediastinum and another invaded into the ribs. Management of hydatid disease is complex and should be based on staging. Improper management can have devastating consequences; increased education in non-endemic regions is needed.

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HIGH DENSITY FERMENTATION OF EG95 IN *PICHIA PASTORIS*

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This study was conducted to explore the expression and high density fermentation of genetically engineered EG95 gene originating from *Echinococcus granulosus*, which causes cystic echinococcosis (CE) in humans and animals with serious public problems and economic loses. The EG95 gene was codon optimized and truncated for expression in *Pichia pastoris*. The genetically engineered EG95 gene was cloned into *P. pastoris* expression vector pPIC9K to construct recombinant plasmid pPIC9K-EG95 (r pPIC9K-EG95). The rpPIC9K-EG95 was then transformed into *P. pastoris* GS115 cells by electroporation, and stable multicopies of recombinant *P. pastoris* strains were selected by G418 resistance with a concentration of 2 mg/L. SDS-PAGE assay of culture broth from one selected expression strain induced by methanol indicated that the recombinant target EG95 protein was about 14.3 kDa and partially glycosylated with the determination of Endo-H. After purification, the recombinant EG95 protein was finally confirmed by mass spectrography. The culture conditions of recombinant *P. pastoris* for its high density fermentation in fermenter were optimized in 4 test batches, and the optimum pH, cultivation temperature and induction time were 5.5, 28℃ and 96 h, respectively. Methanol was added in different adding rate within different fermentation stage. Samples of culture broth were centrifuged (3000×g, 4℃),and the purity and quantity of the recombinant EG95 protein in the supernatant was determined to be 95.9% and 4.68 g/L. In conclusion, the recombinant EG95 protein could be highly expressed in the selected *P. pastoris* strain in fermenters with the stability and high purity. Our performances in this study will contribute to the development of vaccine against CE.

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GEOGRAPHIC CORRELATION BETWEEN TAPEWORM CARRIERS AND HEAVILY INFECTED CYSTICERCOTIC PIGS

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Neurocysticercosis is a leading cause of preventable epilepsy in the developing world. Sustainable community based interventions are urgently needed to control transmission of the causative parasite, *Taenia solium*, to prevent additional neurologic disease. We examined the geospatial relationship between live pigs with visible cysticercotic cysts on their tongues and humans with adult intestinal tapeworm infection (taeniasis)

in a rural village in northern Peru. The objective was to determine whether tongue positive pigs could indicate high risk geographic foci for taeniasis to guide targeted screening efforts. This approach could offer significant benefit compared to mass intervention. We recorded geographic coordinates of all village houses, collected stool samples from all consenting villagers, and collected blood and examined tongues of all village pigs. Stool samples were processed by ELISA for presence of *Taenia* sp. coproantigens indicative of active taeniasis; serum was processed by enzyme linked immunoelectrotransfer blot (EITB LLGP) for antibodies against *T. solium* cysticercosis. Of 548 pigs, 256 (46.7%) were positive for antibodies against cysticercosis on EITB LLGP. Of 402 fecal samples, 6 (1.5%) were positive for the presence of *Taenia* sp. coproantigens. The proportion of coproantigen positive individuals differed significantly between residents of households with a tongue positive pig (2/36, 5.6%), residents living within 100 meters of a tongue positive pig (2/44, 4.5%) and residents living >100 meters from a tongue positive pig (2/322, 0.6%) (p=0.02). The prevalence of taeniasis was >7 times higher among residents living within 100 meters of a tongue positive pig compared to residents living outside this range (adjusted PR 7.5, 95% CI 1.1 52.2). This finding suggests that tongue positive pigs in endemic communities can indicate geospatial foci in which the risk for taeniasis is increased. Targeted screening or presumptive treatment for taeniasis within these high risk foci may be an effective and practical control intervention for rural endemic areas.

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PHARMACOKINETICS AND TISSUE RESIDUE PROFILES OF OXFENDAZOLE IN PIGS

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Oxfendazole (OFZ) is a benzimidazole antiparasitic agent which is effective against pig cysticercosis when given as a single 30 mg/kg dose, and thus a potential key tool in its control/elimination. Only scarce information about its pharmacokinetics and tissue residues in monogastric animals is available. This study assessed OFZ and metabolites [(fenbendazole sulphone (FBZSO2), fenbendazole (FBZ)] plasma pharmacokinetic and tissue residue profiles after a single oral administration to pigs. Two groups of 24 pigs each were assigned to receive a single oral dose of 30 mg/k of either commercial OFZ formulated at 9.06%, or a locally formulated suspension at 22.5%. Blood and tissue samples were collected over 30 days post-treatment and analyzed by HPLC. OFZ was the main compound recovered in plasma, followed by FBZSO2 and low FBZ concentrations. The area under the curve [AUC0-LOQ] of the commercial formulation was 209.9±33.9 µg·h/ml, with Cmax of 5.40±0.65 µg/ml. The parameters for the commercial formulation tended to be higher than those for the locally formulated suspension. FBZSO2 residue levels were the highest found in muscle (0.68±0.39 µg/g) and fat (0.69 ±0.39 µg/g). In liver and kidney the highest residues corresponded to FBZ (5.29±4.36 µg/g) and OFZ (2.86±0.75 µg/g), respectively. In conclusion, high OFZ concentrations over a long period of time complemented with the recovery of the anthelmintically active compound FBZ in the bloodstream are relevant pharmacokinetic data obtained after OFZ administrations at 30 mg/kg, which correlates with the adequate clinical efficacy obtained in pigs infected with cysticercosis. According to the tissue residue profiles, a withdrawal time of 17 days must be allowed for human consumption at this dose

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MOLECULAR DIFFERENCES AMONG ISOLATES OF *TAENIA SOLIUM* CYSTICERCI FROM DIFFERENT REGIONS OF PERU

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Taenia solium cysticercosis is a neglected parasitic disease that is found worldwide. Previously considered to be tool ready for eradication, efforts have not yet proven to be successful. Molecular methods may provide additional insights to better understand the distribution and dynamics *of transmission of T. solium* cysticercosis. We evaluated three loci of *T. solium* as potential genotyping tools. The DNA of the mitochondrial loci for cytochrome B (CyB) and cytochrome C oxidase subunit I (COI), and a nuclear locus encoding the diagnostic antigen Tsol-14 (Ts14) were amplified and sequence analyzed from a blinded set of cysts from 49 different pigs from four distinct geographical regions of Peru. The nucleotide sequences from Ts14 were conserved among all samples, while sequences of CyB and COI showed sequence polymorphisms and unique SNPs. Concatenated pseudogenes were assembled using the sequences of CyB and COI and used to determine phylogenetic relationships among the samples. Neighbor joining tree analyses showed that the sequences clustered into three major clusters A, B and C, while A had 4 sub-clusters (A1-4). After unblinding of the codes we identified that all samples in cluster B were from pigs from the northern coast, and those in cluster C were animals from the southern highlands. Within cluster A, almost all pigs in the sub-clusters A1and A4 were from the central coast, while one pig from A1 was from the central highlands. All samples in sub-cluster A3 belonged to the southern highland (same region as pigs in C), while A2 had one pig from the northern coast and one from the central highlands. These findings suggest that the CyB and COI mitrochondrial loci could be used as preliminary markers for genotyping and better understanding of the transmission dynamics of cysticercosis. Further work would be needed including analyses of nuclear loci as well as samples from other geographical settings.

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FOCAL LOSS OF VASCULAR INTEGRITY AND EOSINOPHIL INFILTRATION ADJACENT TO *TAENIA SOLIUM* CYSTS ARE EXACERBATED BY ANTHELMINTIC TREATMENT IN PORCINE NEUROCYSTICERCOSIS

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The metacestode stage of *Taenia solium* causes neurocysticercosis (NCC) and muscle infections in humans and pigs, with similar cyst morphology. Using the pig as a model of infection in humans, we investigated the effects of treatment with anthelmintics on vascular integrity and host immune reactions to the parasite in the brain and muscles. We used leakage of Evans Blue (EB), infused intravenously, into the tissue around cysts as an indicator of a breakdown of vascular integrity. We examined histopathological changes after anthelmintic treatment following EB infusions in naturally infected pigs. EB leakage was localized to a small region of some cysts appearing macroscopically as a blue dot and indicating a local breakdown of vascular endothelium. On immunofluorescence microscopy, EB penetration into pericystic tissues was identifiable by red fluorescence. Treatment with praziquantel (100 mg/kg po, once) resulted in an increase (8X) in the frequency of muscle cysts with blue dots. Histopathologically, the blue dots correlated with regions of intense cellular infiltrate with abundant eosinophils (Eos) and mononuclear

cells in the cyst wall and surrounding brain or muscle tissue. Interestingly, a significant proportion of tissue Eos had granular blue staining in unstained sections, suggesting uptake of EB dye (presumably bound to albumin). Immunohistochemical staining for an Eos granular protein (EPX) and simultaneous immunofluorescence imaging confirmed that Eos had, indeed, taken up EB in regions of vascular dye leakage. Examination of cyst structure revealed Eos in the tegument, subtegument and the internal regions of the parasites adjacent to the macroscopically identified "blue dots". Taken together, these data suggest a loss of vascular integrity and interaction between the host immune system and parasite occur focally, and that Eos may play a significant role in the pathology that results from this interaction. These findings have important implications for the pathogenesis of histological damage in NCC and may guide strategies for management of the disease.

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FATTY ACIDS METABOLISM AND UPTAKE IN HELMINTHS, FOCUS ON *TAENIA SOLIUM* TSFABP

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Cysticercosis, caused by *Taenia solium*, is a public health problem in developing countries. During characterization of ESTs in the *T. solium* genome project, the most highly expressed mRNA in the adult worm was found to correspond to a fatty acid binding protein (FABP). The FABPs have been involved in the uptake and transport of long chain fatty acids. A recombinant T.solium FABP (TsFABP) was expressed, and its binding affinities determined by spectrofluorimetry; it showed capability to bind different fatty acids with preference for those saturated. The recombinant TsFABP was found to be associated with host-interacting structures in both cysticerci and adult worm in immunochemistry assays, with βTsFABP antibodies being capable of identifying putative homologues in many Taeniid species. Also, while determining the protective potential of the protein, we found specific βTsFABP antibodies in sera from mice infected with *Taenia crassiceps*, suggesting that the protein homologue is exposed to the host in the course of infection. All this results suggest that TsFABP plays a role in the parasite's establishment in host tissues probably involved in the uptake of host's lipids, or its transportation along the syncytial tissues of *T. solium*, before being metabolized. This, supported by the results from the *T. solium* genome project that showed a limited biosynthetic capability in the parasite, lacking enzymes related to fatty acids and lipids modification, v.gr. elongases and desaturases. It is not clear yet how the parasite obtains the necessary fatty acids and lipids for development and maintenance, being apparently unable to synthesize them. The parasite may take up the necessary enzymes from the host, it has been demonstrated already that the cysticerci incorporates important quantities of host´s proteins; or maybe it takes up the lipids directly from the host. Here we explore the potential mechanisms for this probable lipid uptake, using the data obtained from the *T. solium* genome project, and focusing in the probable role of TsFABP.

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ANTIBODIES TO *TOXOCARA CANIS* IN INDIVIDUALS WITH A SINGLE BRAIN ENHANCING LESION

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Neurocysticercosis and cerebral malaria are the most frequent parasitic infections affecting the central nervous system (CNS). Other parasitic infections can also affect the CNS and are likely underdiagnosed because of poor diagnostic suspicion and the lack of accurate diagnostic tests. As an example, neurotoxocariosis is not often considered in the differential diagnosis in patients with brain inflammatory lesions. *Toxocara* is highly prevalent worldwide, can easily reach the CNS, produces granulomatous lesions, and has been associated with neurological symptoms such as seizures. In this study we evaluated archive samples from a series of 101 patients with a single brain enhancing lesion suspected of neurocysticercosis using a western blot assay (LDBIO Diagnostics, Lyon, France) for antibodies to *Toxocara*. From the 101 patients, 56 were seropositive and 45 were seronegative for cysticercosis on western blot. Seroprevalence for toxocariasis was 75.3% (76/101), 56 of 76 individuals reacted to all four diagnostic antibody bands. There was a trend for patients seropositive for cysticercosis to have lower seroprevalence of anti-Toxocara antibodies (39/56 versus 37/45, OR: 0.496, 95% CI: 0.191-1.287, p = 0.145), and cysticercosis seropositive individuals were significantly less likely to react to all 4 antibody bands (25/56 versus 31/45, OR: 0.364, 95% CI: 0.16-0.829, p = 0.015). This unexpected negative association suggests that a proportion of cases of single brain enhancing lesion, seronegative for cysticercosis, may be due to *Toxocara* infections. Alternatively although less likely, patients may have opposite risk factors causing differential exposure, or cross-protective immune responses.

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HIGH PREVALENCE OF SILENT NEUROCYSTICERCOSIS IN AN ENDEMIC RURAL COMMUNITY IN PERU

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Neurocysticercosis (NCC) is a common helminthic infection of the central nervous system and a leading cause of adult-onset epilepsy in low and middle-income countries. However, few population-based studies have examined associations between neurologic symptoms, serology and radiographic findings, particularly as computerized tomography has typically been limited only to symptomatic people. We conducted a population-based neurologic evaluation in a rural endemic village in northern Peru (Rica Playa, Tumbes, pop. 454) to determine the lifetime

prevalence of epilepsy and severe headache in this community. Our 2-stage evaluation began with door-to-door neurologic screening of all residents ≥2 years old using a validated questionnaire followed by clinical evaluation by a study physician for positives. We also collected a blood sample to detect antibodies against *Taenia solium* cysticercosis using an enzyme linked immunoelectrotransfer blot (EITB LLGP). We then invited all residents ≥18 years old, and any person who was seropositive, to have non-contrast computerized tomography (CT) of the head. Of the 385 residents who provided serum, 142 (36.6%) were seropositive on EITB LLGP. Of the 273 residents who accepted CT scan, 53 (19.4%) had radiographic findings consistent with NCC. All 53 had cerebral calcifications (median no. of calcifications 1, IQR 1-2, range 1-15), 1 had a viable cyst, and 1 had colloidal granuloma. Individuals with brain calcifications were twice as likely to be seropositive than those without calcifications (OR 1.9, 95% CI 1.0-3.6). Of the 403 who participated in the neurologic evaluation, we detected 4 residents (1.0%, 95% CI 0.02-2.0%) with epilepsy and 5 (1.2%, 95% CI 0.6-2.3%) with severe headache. Two of the epilepsy cases were active and 2 were inactive. While the prevalence of neurological cases was too small to examine associations, 3 out of 5 (60%) individuals with headache were seropositive and one (20%) had radiographic findings consistent with NCC; none of the examined individuals with epilepsy was seropositive (0/4), nor had NCC compatible findings on CT (0/3, one person with epilepsy refused CT scan). Exposure to *T. solium* is very common in this endemic community where 1 out of 5 residents had brain calcifications; viable forms were rare. However, the vast majority of people with calcifications were asymptomatic.

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WHAT DOES THE ELECTROIMMUNOTRANFER BLOT TELL US ABOUT CYSTICERCOSIS IN PIGS?

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Taenia solium infection burden at village and individual levels remain elusive. This study aimed to relate Electro Immuno Transfer Blot (EITB) seropositivity and pig infection burden. A total of 476 pigs were sampled from a Peruvian endemic area. Seroprevalence was 60.5 ± 4.5% with statistically higher proportion of positive older pigs (>8 months) than young pigs. The logistic model showed that pigs >8 month of age were 2.5 times more likely to be EITB-positive than ≤8 months. A subset of 84 seropositive pigs was necropsied. Forty-one out of 84 positive pigs were negative to necropsy (48.8%) and 43 (51%) had one or more cysts. The Generalized Estimating Equations (GEEs) were applied to fit an ordinal logistic regression model on the necropsied pigs. The model accounted for household clustering since we had to purchase more than one pig in some houses. The probability of having moderate or heavy infection burden increases proportionally with the number of EITB bands. We also demonstrated that there is a high probability of being necropsy-negative for pigs having less or equal than three EITB bands. Therefore, the EITB might be a measure of exposure rather than a test to determine the real prevalence of cysticercosis infection.

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TAENIA SOLIUM : DEVELOP OF A RAT MODEL OF NEUROCYSTICERCOSIS

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Neurocysticercosis is the most common parasitic disease of the central nervous system (CNS) caused by the metacestode form of the tapeworm *Taenia solium* worldwide. The development of a rat model of Neurocysticercosis (NCC) with *Taenia solium* oncosphere was undertaken to: i) obtain a suitable experimental model for neurocysticercosis, ii) describe the inflammatory process, infection, and iii) describe the host-parasite relationship in rat. The rat Holtzman were intracranially inoculated with oncospheres of *T. solium* at different concentrations (10, 20, 30 and 40 parasites) to induce NCC. They were euthanized at 90 days after the inoculation. Their encephala were removed for the histopathologic analysis. Observations of 90 dpi, included the presence of cysticerci in the brain of the rat in the macroscopic observation, specifically in the rats that were inoculated 20 and 30 parasites. Observations which were corroborated by Westrern blot. and histology. In conclusion, this model resembled the natural infection of NCC using the *T. solium* oncosphere as natural stage of infection and the same species of parasite that causes the NCC. It will aid (i) in understanding disease progression, from the early to late stage of infection (ii) improving and generating new diagnostic tools, and (iii) refining or rationalizing the current treatment of the disease in humans.

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GENETIC DIFFERENTIATION OF *TAENIA SOLIUM* STRAINS FROM DIFFERENT PERUVIAN COMMUNITIES USING MICROSATELLITES

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Cysticercosis, endemic to several developing countries, is a neglected helminthic disease that disproportionately affects people, causing significant chronic neurological disease. In contrast to other taeniid cestodes, knowledge about genetic variation in *Taenia solium* is lacking. This is an important consideration in the epidemiology and transmission of these parasites, since genetic variants may differ in their infectivity, pathogenicity and response to treatment. Until now the studies on genetic variability have been based on molecular markers, such as RAPD, and showed poor diversity. Therefore, it is required a tool of sufficiently high resolution to differentiate strains. The availability of the *T. solium* genome let us demonstrate that microsatellites sequences are broadly distributed in coding and no coding regions. Using bioinformatics tools we identified several microsatellites sequences. Microsatellites were identified from a single locus using Blast and an ad-hoc script developed by Gur-Arie et al. Repetitive motifs of 3-10bp with a minimum of three repetitions were filtered. The markers that showed differences in the motifs between the genomic sequence and the *T. solium* ESTs published in the GenBank. Thirteen markers were selected and further evaluated. These markers

were tested with a sample of 12 tapeworms collected from two distant regions in Peru. Six tapeworms were from Tumbes and six from Puno. Each candidate marker was individually amplified from parasite DNA by polymerase chain reaction. The products were analyzed using the QIAxcel System. The size of the amplified product was used for identification. All fragments were amplified and showed the expected theoretical size. Three of them were polymorphic, SSR1, SSR6 and SSR7. These markers were able to differentiate tapeworms from Tumbes and Puno, which were clustered in two groups. Microsatellites polymorphism showed that *T. solium* has genetic diversity not previously reported, supporting the hypothesis of a genetic recombination process in *T. solium*.

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SPATIAL DISPERSAL OF DENGUE IN TWO URBAN AREAS OF SOUTHEAST ASIA: TESTING MULTIPLE MECHANISTIC MODELS

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Dengue has been endemic in Southeast Asia for decades. In much of the region individuals will regularly suffer twice from the disease by the end of their teenage years. Prevention measures, including the use of insecticides and the targeted destruction of oviposition sites has been hampered by poor understanding of the local dispersion of the virus, especially the relative contributions of human and mosquito movements. To address this gap we collaborated with local hospitals and ministries of public health in Bangkok, Thailand and Cebu in the Philippines to develop models to understand the local spread of the virus. We used the geocoded spatial location of 18,425 patient homes who became sick with dengue between 1994 and 2010. We found a significant risk of finding a second case within a month and up to one km of an initial case relative to that expected given the underlying spatial and temporal distribution of cases. In addition we found a steady increase in the extent of spatial dependence between cases over the time series, potentially indicating increasing mobility of individuals. We used population density estimates from LandScan to build agent based simulations of various parametric and nonparametric dengue transmission processes in both cities. We demonstrated that a gravity model was most consistent with the observed patterns of spatiotemporal dependence with a mean distances of under 100m between sequential cases in a transmission chain. These findings provide key insight into the potential dispersal mechanisms of the disease and provide a guide for the targeting of interventions upon identification of dengue cases.

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DECONSTRUCTING THE PROTECTIVE IMMUNE RESPONSES ELICITED BY A NOVEL DENGUE TETRAVALENT VACCINE IN AG129 MICE

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A formulation of a chimeric dengue vaccine viruses containing the pre-membrane (prM) and envelope (E) genes of serotypes 1-4 expressed in the context of the attenuated DENV-2 PDK-53 genome (DENVax) was tested for immunogenicity and efficacy in interferon receptor knock-out AG129 mice. The vaccine was highly immunogenic and elicited protective immune responses against both wild-type (wt) DENV-1 (Mochizuki strain) and DENV-2 (New Guinea C strain) challenge viruses. To understand better the contribution of each chimeric vaccine in the induction of immune responses and protection afforded by DENVax monovalent formulations were injected in AG129 mice and were shown to elicit robust neutralizing antibody responses to the homologous virus and only limited cross-reactivity to other serotypes. A single dose of monovalent DENVax-1, -2, or - 3 vaccine provided eighty or greater percent protection against both wild-type (wt) DENV-1 and DENV-2 challenge viruses. A single dose of monovalent DENVax-4 also provided complete protection against wt DENV-1 challenge and significantly increased the survival times after challenge with wt DENV-2. Preliminary studies from passive transfer of immune serum suggest a potential role of humoral immunity in protection. Currently, we are evaluating the role of $CD4^+$ and $CD8^+$ T cells in protection against homologous or heterologous DENV challenge. Overall, these data highlight the immunogenic profile of DENVax, a novel candidate tetravalent dengue vaccine that is currently in phase I and II clinical trials. In addition, a better understanding on how DENVax works would greatly facilitate our efforts in developing more effective vaccination strategies against dengue.

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CHALLENGES TO DENGUE REPORTING AND SURVEILLANCE IN TRINIDAD AND TOBAGO: LABORATORY DIAGNOSTICS IN THE ABSENCE OF SUFFICIENT CLINICAL DATA

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The Trinidad Public Health Laboratory (TPHL) receives sera from suspected dengue cases for confirmatory testing by a commercially available dengue-specific IgM ELISA. Samples are typically subjected to point-of-care screening at the sending institutions but are often received by TPHL with incomplete laboratory request forms e.g. no record of number of days post onset of illness (d.p.o.), symptomology or demographics, all crucial for determining the most appropriate screening procedure and generating beneficial surveillance data. We examined the challenge presented to TPHL by reviewing the data accompanying a batch of sera (n = 500) received during the 2011 dengue outbreak, which were also screened by a commercially available NS1 Antigen Capture ELISA kit. Of these, 94% were accompanied by point-of-care screening results. The proportions with other data were: sex 62.2%, age 31.4%, patient address 7.2%, d.p.o 3% and symptomology 9.8%. TPHL screening found 45% IgM+/NS1+, 18% IgM+/NS1-, 11.8% IgM-/NS1+ and 21.4% IgM-/NS1-. Of 19 with inconclusive IgM results, 10 were NS1+ and 9 NS1-. Thus NS1 testing detected 69 positive sera that would have been reported as negative or inconclusive. 18 of these were tested by reverse transcriptase PCR and the presence of dengue virus RNA was confirmed in 11. Using TPHL IgM and NS1 ELISAs as gold standards, the sensitivity and specificity of point-of-care IgM screening were 92.3% and 30.9% respectively, and for point-of-care NS1 screening, 85.5% and 90.9% respectively. Our data highlight the benefit of combining virus specific direct testing methods with indirect testing methods when there is inadequate clinical data. However, due to the number of cases with insufficient data, cost effectiveness would be a concern. For improved efficiency and accuracy of dengue reporting and surveillance in T&T, procedures addressing deficiencies in data gathering and screening services must be adopted at all institutional levels. Additionally the low specificity of the point-of-care IgM screening deserves further investigation.

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THE EFFECT OF ANTIBODIES ON ENDOTHELIAL CELLS DURING DENGUE VIRUS INFECTION

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Primary infection with one Dengue virus (DENV) serotype is proposed to confer lifelong homotypic immunity, but only short-term heterotypic immunity to the other three serotypes. Secondary heterologous infection can result in Dengue Hemorrhagic Fever or Dengue Shock Syndrome (DHF/DSS). Evidence supports a role for pre-existing antibody (Ab) to Dengue virus in DHF/DSS pathogenesis via antibody dependent enhancement (ADE), in which Abs from the initial infection enhance virus infectivity rather than neutralize leading to increased virus uptake into cells. Increased vascular permeability caused by loss of endothelial cell (EC) barrier integrity is a hallmark of DHF/DSS in humans. However, the extent of EC permissiveness to DENV infection and the degree of cell death resulting from DENV infection is controversial, as is the mechanism of hemorrhage associated with DENV infection. We hypothesize that DENV Abs can enhance EC infection leading to loss of EC barrier function and vascular leak syndrome. Anti-DENV human monoclonal antibodies (HMAb) were generated by molecular cloning and characterized based on DENV neutralizing and/or enhancing properties *in vitro*. Human dermal microvascular ECs (HMEC-1) were infected with DENV pre-incubated in the presence or absence of HMAbs known to enhance infection. RNA extracted from infected HMEC-1 cells and supernatants were used to quantify DENV genome copies within the cells and released by the cells, respectively, by qRT-PCR. Infectious virion production was determined by plaque assay. HMEC-1 cells supported direct DENV infection, in the absence of Ab, and enhanced infection in the presence of HMAb, demonstrated by increased viral genome copies within cells. These studies will determine the precise role of DENV Abs in enhancing EC infection and inducing EC pathology. Characterizing the ability of HMAbs from DENV infected individuals to induce EC pathology *in vitro* will provide an understanding of the role of Abs in the induction of DHF/DSS.

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OPTIMIZATION AND VALIDATION OF THE PLAQUE REDUCTION NEUTRALIZATION TEST FOR THE DETECTION OF DENGUE VIRUS NEUTRALIZING ANTIBODIES USED IN SUPPORT OF DENGUE VACCINE DEVELOPMENT

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A reliable testing method that properly measures the immune responses to natural infection and/or vaccination is imperative for public health surveillance and vaccine evaluation. It is anticipated that the neutralizing antibody response against each dengue virus serotype may correlate to protection, and the plaque reduction neutralization test (PRNT) is recognized as the gold standard to measure dengue virus neutralizing antibodies. A factorial design of experiment (DOE) approach was used for the development and optimization of a dengue $PRNT_{50}$ in consideration of WHO guidelines. To generate a robust test method, critical testing parameters included optimal number of days of cell seeding prior to performing the assay, percentage of overlay medium and days of incubation post-infection to generate a robust assay method were evaluated and defined. The optimized $PRNT_{50}$ method was then validated in accordance with International Conference for Harmonization (ICH) guidelines. Intra-assay and inter-assay precision of the dengue $PRNT_{50}$ demonstrated that the titers for 87.5-100 % (14/16 -16/16) and 95-100% (19/20-20/20) of the samples tested for all 4 serotypes were within 3-fold dilution of the median titer. Suitable accuracy and dilutability were demonstrated across targeted dilution (1:4, 1:16, and 1:64) for all 4 serotypes. Assay specificity for each of the 4 serotypes was shown by selectively measuring dengue serotype-specific neutralizing antibodies in the presence of other Flavivirus antibodies that were spiked into the test serum samples. The LLOQ of the assay was challenged and assessed by testing the low titer samples repeatedly 11 times for all 4 serotypes. In summary, the validated dengue $PRNT_{50}$ was demonstrated to be suitable to detect and measure the level of dengue virus serotype-specific neutralizing antibodies in human serum samples with acceptable intra-and inter-assay precision, accuracy/dilutability, specificity, and with a LLOQ titer of 10. This assay is being used to support the worldwide clinical development of the CYD dengue vaccine.

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DEFINITION OF PROTECTIVE VERSUS PATHOGENIC IMMUNE PROFILES AFTER DENGUE VIRUS INFECTION

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As the four serotypes of dengue virus (DENV-1-4) increasingly co-circulate, the risk of severe manifestations of dengue disease is of greater concern. Although half of infections are estimated to be subclinical, nearly one-third of the world's population is at risk of DENV exposure. Through a unique cohort study in Thailand, we were able to obtain blood samples from school-age children before and after the dengue season. A four-fold increase in anti-DENV antibody titer was used to identify individuals who experienced DENV infection during the study. Individuals were further stratified into those who experienced overt dengue illness and those who seroconverted without presenting symptoms. This latter outcome, protection from clinical disease, is one goal of vaccination. Since dengue pathogenesis is thought to be linked to an aberrant immune response, we sought to define the balance between protective and pathological immunity using an *in vitro* stimulated PBMC culture system and a multiplexed analysis of cytokine and chemokine content in supernatants. Pre-exposure PBMC were isolated from fifty-one individuals who subsequently had either subclinical or symptomatic infection. After five days in culture with DENV isolates representing the four serotypes, culture supernatants were harvested and subjected to a multiplexed, bead-based array to quantify the presence of 30 different cytokines and chemokines. The authors will show serotype-specific cytokine levels for individuals according to clinical outcome. Studies comparing individuals with varying clinical outcomes are critical for defining protective versus pathogenic immune profiles. Previous studies have shown that neutralizing antibody titers are a poor correlate of protection from dengue disease in this highly exposed population. These analyses of *in vitro* cytokine responses contribute to the understanding of immunity in natural DENV infection and should help inform future vaccination strategies.

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THE ECONOMIC AND DISEASE BURDEN OF DENGUE IN SOUTHEAST ASIA

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Dengue poses a substantial economic and disease burden in Southeast Asia (SEA). Quantifying this burden is critical to inform policy makers, set policy priorities, and implement disease-control strategies. The few published estimates of dengue burden in SEA are based on one or a few countries and their generalizability is limited by methodological differences. We addressed this need by estimating the economic and disease burden of dengue in 12 countries in SEA, containing 560 million

(m) people. We obtained reported cases from multiple sources, including surveillance data, WHO, and published studies. Underreporting was adjusted using expansion factors (EFs)--multiples of reported numbers--obtained from previous empirical studies in SEA. We conducted a systematic literature review to obtain the direct and indirect costs per dengue episode, by country. We extrapolated unit costs using linear regressions with GDP per capita and type of treatment as independent variables, to complete data for countries with published studies. We estimated the cost per fatal episode based on productivity loss using GDP per capita and life expectancy, and used WHO methodology to estimate disease burden (DALYs). As sensitivity analyses, we used 1,000 Monte Carlo simulations varying EFs, share of hospitalized cases, unit costs, and DALYs per case. We obtained an annual average (years 2000-2010) of 3m dengue cases (1m hospitalized and 2m ambulatory), 6,994 deaths, and a weighted overall EF of 8.4. The annual economic burden (with 95% certainty level) was US$896m ($401m-1,920m) or US$1.60 ($0.72-3.43) per capita. The annual number of DALYs lost from dengue was 292,000 (129,000-612,000), or 521 (230-1,092) DALYs per m inhabitants. Cost per capita was 66% of that previously found for the Americas, but DALYs per m were 6.4 times as high. The burden of dengue would be higher had we considered other economic costs, such as prevention and vector control, and long-term sequelae of dengue. These results suggest that efficient technologies that reduce burden of dengue would be cost effective.

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SEROPREVALENCE OF DENGUE IN UNITED STATES SPECIAL OPERATIONS COMMAND PERSONNEL DEPLOYED TO DENGUE-ENDEMIC AREAS

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Both the endemicity and clinical sequelae of dengue infection are increasing worldwide. The increases are at least in part a result of widespread travel and the increased range of *Aedes albopictus*, a competent vector of dengue. US Army Special Operations Command (USASOC) personnel are at an increased risk of exposure to dengue based on their widespread presence in dengue endemic areas worldwide. Furthermore, repeated deployments to these areas, oftentimes with the same personnel going to different dengue endemic areas sequentially, increase the risk for developing the more serious sequelae of dengue, dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). Information about the seroprevalence rate of dengue in USASOC personnel is critical to assessing risk to this population for future deployments and tailoring preventive medical countermeasures and leveraging field diagnostics. In the first part of a two part project to assess baseline seroprevalence of dengue in USASOC units, a random, unit-stratified sample of 500 anonymous serum specimens from personnel assigned to units in USASOC deployed to Latin America from 2006-2008 were screened for dengue antibodies using a microneutralization assay. Of the 500 specimens screened, 56 out of 500 (11.2%) had neutralizing titers (MN50 ≥ 10) against at least one DENV serotype; subsequent positive sample titration resulted in 48 out of the 56 positive samples (85.7%) with NT titers (MN50 ≥ 10) against at least one dengue serotype for an overall dengue exposure rate of 9.6% (48 of 500). Similar results (15/111, 13.5%) were found on subsequent predeployment testing of USASOC personnel in 2012. These findings show that exposures to dengue in USASOC operations are more common than previously thought thus lending increased importance to preventive countermeasures. The significance of these findings with regards to force deployment and personal risk is discussed.

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A MODEL OF TRANSDISCIPLINARY STUDY DESIGN FOR UNDERSTANDING DENGUE TRANSMISSION IN THE DEVELOPING WORLD: APPLICATION OF AN ECOHEALTH APPROACH IN BANGLADESH

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A marked global re-emergence of epidemic dengue reflects the failure of interventions based on traditional reductionistic disciplinary approach to the understanding of dengue disease transmission. By challenging these reductionist notions, the present study asserts the notion that comprehending dengue disease transmission requires application of a holistic, transdisciplinary epistemology that can assess the driving eco-bio-social determinants and their interaction with human action. Developing an appropriate transdisciplinary research design in the context of developing countries requires bringing the knowledge and practice partners together (vertical integration), along with integration of multiple disciplinary areas (horizontal integration), and considerations of social, economic and cultural context without compromising scientific goals. We formulated a multi-tier transdisciplinary study design to apply a holistic "Ecohealth Approach" to understand dengue virus transmission and the dynamics of human choices and preferences concerning this. The study was implemented in Dhaka, Bangladesh, where the 16 million occupants have been exposed to a resurgence of dengue since 2000. To develop a suitable research design, we considered variation in: socio-economic status among the city-zones, gender inequality, population density, housing, and water supply, waste disposal and sewage systems. Multiple disciplinary aspects were encapsulated by examination of: i) rates of human exposure to dengue virus (DENV) by identifying individuals (via a serosurvey in 1200 households) with IgM and IgG antibodies to DENV and acute cases of illness from hospitals (200 diagnostic study of suspected hospitalized patients) by identifying the presence of DENV RNA by PCR amplification procedures; ii) abundance of dengue vector (*Aedes aegypti* and Ae. albopictus) during monsoon and dry seasons in the same households; iii) self-risk perception by the community members, and other patterns of human behavior; iv) human population density, available housing and water and waste supply and disposal systems; and iv) human organizations responsible for interventions. We envision that such a transdisciplinary, holistic epistemological framework will help to determine the eco-bio-social factors responsible for dengue virus transmission, and which can then be apply to the general context of the developing world.

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DEVELOPMENT OF A TARGET PRODUCT PROFILE FOR DENGUE DRUGS

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Dengue fever has serious public health implications for Florida and currently threatens nearly 3 billion people worldwide. The USF Global Health Infectious Disease Research Program (GHIDR), USF Center for Drug Discovery and Innovation (CDDI), Florida's Schools of Pharmacy at UF and USF, and the UF Emerging Pathogens Institute (EPI) have developed a consortium for novel solutions for the detection, prevention and treatment of vector borne diseases. Critical to the consortium's success has been the ongoing development of an initial Target Product Profile (TPP) for potential dengue anti-viral drug candidates. The ideal drug candidate should be active against all known dengue strains, used in primary and secondary infectious independent of serotype, reduces progression to hemorrhagic disease, have a low cost of goods, be fasting acting, rapidly reduce viral burden, and have a safe clinical profile. With no current treatments and only supportive care available, there is no gold standard. Creation of a

unique road-map to druggability represents a daunting challenge. TPPs give researchers a better understanding of existing and future unmet needs for control or possible eradication of dengue. By 'beginning with the end in mind' TPP planning allows for all working areas (e.g. preclinical, clinical, business marketing, distribution and access) to discuss the necessary features critical for end use and reach an initial consensus on the approaches to the milestones and objectives of drug discovery and development. Applicable in all areas of pharmaceutical development, TPPs have the potential to be of even greater impact in tropical neglected diseases.

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VALIDATION OF SURVEY FOR MOSQUITO AVOIDANCE PRACTICES IN INTERNATIONAL TRAVELERS FOR DENGUE PREVENTION

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Dengue is the most common Arboviral infection in travelers to international destinations in tropical and sub-tropical environments. Currently, there is no vaccine or treatment for Dengue; rather, travelers are offered a set of mosquito avoidance practices (MAP) as recommendations for decreasing the risk of infection. In addition, travelers who are 'visiting friends and relatives' (VFR) have a risk of acquiring Dengue equivalent to those living in endemic regions, and an increased risk of severe Dengue if infected during travel. A pilot study was conducted to identify the factors associated with compliance to the MAP guidelines in travelers planning to VFR. A mixed methods survey was developed to correspond with the Precaution Adoption Process Model (PAPM) and tested in travelers to the cultural celebration, Carnival, in Trinidad & Tobago 2012. The survey successfully measured Dengue knowledge, attitudes, intent, motivation and cultural influence on planned travel behavior for MAP. Construct validity and reliability of the instrument was determined by performing an exploratory factor analysis. The resultant Cronbach alpha was .94, indicating internal reliability. The constructs which loaded in the factor analysis were in agreement with the theoretical models developed to investigate the factors associated with compliance to MAP and the PAPM-Dengue model. Further research is necessary to determine if the theoretical models can be used to predict travel behavior; however, the survey can be used as a tool to determine the stage of a traveler within the PAPM-Dengue model. The stages of the model indicates the traveler's level of awareness, perception of Dengue issues, motivation for Dengue prevention at the individual level, and attempts to compliance with MAP recommendations during travel to endemic regions. This can aid in better understanding the social epidemiological aspects of Dengue transmission in travelers.

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DENGUE AND PREGNANCY IN KAMPHAENG PHET PROVINCIAL HOSPITAL

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Dengue, the vector- borne disease is a major problem particularly in tropical region like KPP, Thailand. About 60% of dengue is reported in adults during past few years and the pregnant women are susceptible to dengue infection with high mortality rate. Dengue and pregnancy is rarely reviewed and summarize in Thailand. KAVRU provides the dengue PCR testing in admitted patient at KPPPH for 20 years that help in diagnosis and early treatment. This retrospective review aims to describe the clinical and laboratory data in confirmed cases of dengue and pregnancy. 16 pregnant were diagnosed of dengue from ICD-10 during 2009-2011 but only 13 cases had confirmed dengue infection either by PCR testing or IgM and IgG ELISA level. The result showed the average age of the patient is 22.7 years (range 16-33), mostly are housewife and employee (38% for each group) and in the 3rd trimester of pregnancy (62%), admitted duration after illness onset range from 1 to 6 days. The clinical presentation are Fever (100%), Muscle/Body pain and Malaise/Fatigue (85%), Headache, loss of appetite and skin rash (70%), Nausea(62%), Vomiting and abdominal pain (54%), others are cough (46%), Joint pain (38%), sore throat, diarrhea and eye pain (23%), Three of the patients were bleeding (2 -epistaxis and 1 melena) . None has neurological involvement. The 2 hDF, 3 DHF I, 5 DHF II and 3 DSS were classified. The PCR on admission day revealed 3 DENV-1, 6DENV-2, 1DENV-4 and 3 negative. The serologies were 10 acute secondary dengue infection. Three of the patients were in labour; 2 were normal labour and 1 was cesarean section due to twin pregnancy. All infant had good Apgar score without fever. From this data, we can summarize that the warning signs in non-pregnant and pregnant are the same but obviously the most symptoms in pregnant are fever with muscle pain and malaise more frequently than gastrointestinal symptoms and this can imply to guideline for dengue with pregnancy and lead to vertical transmission detection.

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DENGUE EPIDEMIOLOGY AND ASSOCIATED FACTORS IN RICE FARMERS OF ENDEMIC REGION OF PERUVIAN AMAZONIAN

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Dengue fever is an endemic infection in the Peruvian Amazonian and has become a public health problem. We conducted a seroepidemiological cross-sectional study in rice farmers of the Alto Mayo Valley. The objective was to determine the prevalence of IgG and IgM antibodies against dengue and to identify the factors associated with positive serology. 250 farmers were enrolled, the prevalence of IgM antibodies (recent infection) was 7.25% (95%CI: 3.79-10.6). IgG antibodies (past infection) prevalence was 62.4% (95%CI: 56.2 - 68.6). None of the participants with recent infection presented suggestive symptoms of dengue. Only two of the 18 participants with recent infection had headaches, sore calves, back pain, abdominal pain and 3 had pain joints. However, the presence of these symptoms was not associated with dengue infection. In bivariate analysis, farmers older than 30 years (OR= 2.52; 95%CI: 1.22-5.22; p=0.01), residence time more than 25 years in the area (OR=2.19; 95%CI: 1.29-3.72; p=0.004) and farmer working more than 15 years (OR=1.83; 95%CI: 1.06-3.18; p=0.003) have associated with past infection. Age, gender, years of residence in San Martin region, floor material of house, store water inside the home, bird breeding, home proximity to drains, time of work as farmer and elevated floor from the ground were included in the multivariate analysis. Only the residence time of more than 25 years in the region was associated (adjust OR=1.88; 95%IC: 1.04-3.38). In conclusion; dengue virus is circulating in the region affecting more than half of enrolled farmers. The infection is mainly asymptomatic in acute cases. Time of residence in this region for more than 25 years was associated with past infection of dengue, which suggests the existence of multiple factors or cultural, home and works characteristics and that they have not been explored in our research.

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EVALUATION OF THE PERFORMANCE OF CLINICAL AND LABORATORIAL DENGUE DIAGNOSIS DURING AN EPIDEMIC IN A MEDIUM-SIZED CITY IN SOUTHEAST BRAZIL

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Acute dengue disease may present with symptoms that overlap with other febrile diseases, making it impossible to reach the diagnosis based solely on clinical grounds.The WHO definitions indicate that a probable dengue case is characterized by fever and two of the most common dengue symptoms, such as rash, aches and pains, and a positive tourniquet test, and a confirmed case as the one fulfilling the above mentioned clinical criteria associated to virus isolation, a positive PCR, and either IgM or IgG seroconversion in paired samples. However, since viral isolation and performing PCRs need specialized laboratories, most of the time the dengue diagnosis is performed by detecting either, or both, NS1 antigen and dengue-specific IgM antibodies. In Brazil, when dengue incidence rates are over 300 cases/100,000 habitants, dengue cases are no longer confirmed by laboratory tests and every clinical suspected cases are confirmed as dengue cases if they fulfill the WHO criteria for the probable dengue case. In order to assess the performance of the clinical-based diagnosis in epidemic situations, a convenience sample of clinically diagnosed dengue cases were blindly tested by NS1 antigen and dengue-specific IgM antibody detection, according to the manufacturers' protocols. These acute phase samples (up to 5 days of disease onset) were collected on the last week of April and first week of May of 2011 in Ribeirão Preto, a city located in Southeast Brazil and that experimented a huge dengue-1 outbreak, with 21,142 confirmed dengue cases, most of them based on the clinical manifestations. A laboratory confirmed dengue case, used as gold standard for the diagnosis, was defined when a sample was positive for either NS1 antigen or IgM-antibody detection alone or when both were positives. Out of 1,490 suspected dengue cases, dengue diagnosis was determined in 1,148 and in 969 by clinical/epidemiological and laboratory evaluation, respectively. The sensitivity, specificity, positive and negative predictive values were 82.6%, 33.4%, 69.8% and 50,9%, respectively. Thus, it is clear from this work that a clinically-based diagnosis is highly sensitive but it is not very specific, resulting in an overestimation of the real dengue cases. Finally, this study shows that a more reliable dengue-specific test is urgently needed, even though recently, NS1 antigen detection has been used to confirm dengue cases during the acute phase of the disease.

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DENGUE VIRUS INFECTION OF U.S. MILITARY SERVICE MEMBERS FOLLOWING DEPLOYMENT TO DENGUE ENDEMIC REGIONS

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Dengue virus is the most important arthropod-borne viral disease worldwide and a significant infectious disease threat to deploying military personnel. Although it has been found to be a cause of febrile illness and lost duty time since the Spanish-American War, the epidemiology of infections in contemporary worldwide military missions has not been described. To characterize the risk of dengue infection on deployment, pre- and post-deployment banked serum from 1000 Service Members who deployed to dengue endemic regions were tested for the presence of neutralizing antibody against all four dengue virus serotypes to identify Service Members who were infected during deployment. A 7.6% global seroprevalence was found post-deployment; regionally, 12.4% prevalence was found among deployers to South America, 7.2% to Southeast Asia, 6.0% to Africa, and 4.8% to Central America. Odds of experiencing fever during deployment were more than three times greater among those with dengue antibodies post-deployment than those without. Demographic data from post deployment questionnaires, to include age, gender, branch of military service, and military occupation, were analyzed for association with dengue seroprevalence. Age was found to be a risk factor, whereas combat and law enforcement occupations were found to be protective. These results confirm that dengue infection is a military threat in our current theaters of operation. Further, this will help guide medical threat planning and better describe risk factors for infection among adults from non-endemic populations deploying to endemic regions.

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EVALUATION OF THE PERFORMANCE AVAILABLE DENGUE DIAGNOSTIC TESTS: PROGRESS TOWARD AN OPTIMAL DIAGNOSTIC ALGORITHM

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Dengue diagnostic testing has been problematic because no single test detects a single diagnostic analyte with the sensitivity required to confirm the diagnosis using a single specimen obtained during the febrile phase of the illness. However, use of two diagnostic tests; one to detect dengue virus (DENV) RNA or antigen (NS1), and one to detect IgM anti-DENV confirms a high proportion of persons with dengue. This study was conducted to provide an estimate of the performance of dengue diagnostic tests during the first 14 days after onset of fever and to develop a diagnostic testing algorithm for single specimen testing. The longitudinal course of the illness was reconstructed using a panel (n=1234) of archived acute and convalescent serum samples obtained from previously confirmed dengue cases on days 0-14 days after their onset of symptoms (DPO). The panel contained all four DENV serotypes and cases occurred in Puerto Rico between 2005 to 2010. Specimens were tested by CDC developed qRT-PCR and IgM anti-DENV tests and commercial ELISAs for NS1 antigen and IgM anti-DENV. The results from this evaluation provided the most robust DENV laboratory testing algorithm in which end-users have a comprehensive set of results of test sensitivity, specificity and confidence intervals in relation to DPO. In addition, an analysis of the sensitivity of these tests in relation to one another (i.e. qRT-PCR sensitivity in serologically confirmed cases; or IgM sensitivity in qRT-PCR-positive cases by serotype) was determined.These results will be used to develop an algorithm of diagnostic testing to provide the highest likelihood of a definitive result in a single serum/plasma specimen obtained during the acute phase of dengue (DPO= 0-14) in persons with dengue as defined by the 2009 WHO Dengue Case Classification.

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IMPLICATIONS OF POPULATION STRUCTURE FOR GENETIC STUDIES IN DENGUE

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Dengue virus infection is globally the most common mosquito-borne infection after malaria, although only a small percent experience the more serious form of disease. Recent studies in Thai and Taiwanese pediatric populations demonstrated a strong association of dengue hemorrhagic fever (DHF) with the gene CD209, and with JAK1 in a Brazilian largely

adult population from Salvador (n=522). Also, the gene IL28B has been shown to be strongly associated with clinical presentation and treatment outcome of the flavivirus, hepatitis C. Despite laboratory and literature support, none of these genes were associated in a recent GWAS study of dengue shock syndrome (DSS) in Vietnam. In order to test these candidates in a different Brazilian population, 620 subjects from the city of Fortaleza (1000 km north of Salvador) were genotyped at 730 SNPs in the type I IFN response pathway genes and other potential candidates. Both samples were community-based and mirrored the census characteristics for adults in these cities. We failed to see an association for any of the 3 candidates with DHF. Further analysis revealed a surprising degree of city-specific population structure despite the high degree of admixture in Brazil. We performed analysis with the program Structure as well as principal components analysis (PCA) to determine the proportion of contribution from African and European ancestry for all individuals based on pseudoancestors from the HapMap database. Data sets for Native Americans were not available to us. Although both cities are administratively considered The Northeast, we found that they demonstrated 3-fold difference in African genetic contribution (Salvador>Fortaleza) and behaved as distinct populations. Using genomic control (GC) analysis, we found that the population homogeneity was greater for Fortaleza than Salvador, though both had β(inflation factor) values near 1. Values close to 1 indicate little structure. When analyzed together, βwas 1.4. In the Americas where admixture is the rule rather than the exception, studies may fail to find true associations (or find spurious ones) even in the same geographic region or country because of stratification. Unless the gene effect is very large and the mutation very old, association across widely separated populations will be difficult to establish. Either some of these associations are spurious, are of small effect or there are population- and age-specific genetic associations.

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HOW CAN THE TRENDS IN INCREASING SEVERITY OF DENGUE EPIDEMICS BE REVERSED - TAIWAN'S EXPERIENCES TO GLOBAL CONTROL

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Although epidemics of dengue/dengue hemorrhagic fever (DHF) have occurred more than about sixty years since the outbreak of DHF exploded in the Philippines, the severity of dengue epidemics involving many DHF and fatal cases have increased in recent two to three decades in Asia and South American countries. The reasons to this trend in increasing severity of dengue epidemics include global warming, increases in rapid travel, mosquito breeding sites and population movement, failure in effective prevention and control and the selection of dengue virus with greater epidemic potentials. However, what are the mechanisms involved in this increasing epidemic severity through population levels and the selection of more virulent dengue viruses (DENV) over time in the same epidemic in one area or cross-country spread at molecular and cellular levels have remained unclear. In Taiwan, most epidemics of dengue started from imported dengue viruses. However, DHF cases only occurred in certain years. Since dengue has not become endemic/hyper-endemic yet, most epidemics are caused by a single serotype of dengue virus, and each suspected dengue case has to be reported with specimen taken and laboratory confirmation, all these together provide the best chance to understand the epidemiological characteristics between mild clinical form (dengue fever, DF) versus severe clinical form (dengue hemorrhagic form, DHF). We have used geographical information system (GIS) and molecular investigation to find the changes of quasispecies of DENV population in different cases within a family over time. Recently, we examined both dynamic changes of viral and immunological attributes along the epidemics and found that both viral and immunological factors are involved in the selection of DENV through a series of interactions between DENV and host responses.

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THE POTENTIAL ECONOMIC VALUE OF A DENGUE DRUG

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There are currently no approved drugs or vaccines to treat dengue fever. Drugs that prevent the progression of uncomplicated dengue to more severe disease (DHF and DSS) have the potential to significantly reduce morbidity and attendant health care costs. While a tetravalent dengue vaccine is likely to be approved in 2015, and this will be an important advance in the prevention of dengue, it is unlikely to result in the cessation of dengue transmission in the short term. Dengue drugs, if they become available, will offer a complementary approach to the management of the disease. This presentation will, for the first time in the public domain, describe the global economic burden of dengue, the likely impact of vaccine approval on future dengue case loads, the potential approaches to pricing and the future market for a dengue drug.

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IMMUNOGENICITY AND SAFETY OF INACTIVATED CHROMATOGRAPHICALLY PURIFIED VERO CELL-DERIVED JAPANESE ENCEPHALITIS VACCINE IN CHILDREN

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Japanese encephalitis (JE) is a common cause of viral encephalitis in Asia which can be controlled by safe and effective vaccines. Inactivated mouse-brain derived vaccine has a worrisome safety profile while live attenuated vaccine cannot be used in immunodeficient individuals. This study aimed to evaluate the immunogenicity and safety of inactivated chromatographically purified Vero cell-derived JE vaccine (ICVJEV, Beijing P-3 strain) among healthy children. One hundred and fifty-two healthy Thai children aged 1-3 years with no history of JE vaccination received 3 doses of ICVJEV (Liaoning Cheng Da Biotechnology Co., Ltd, China) on Day 0, 7-28, and 365. JE virus neutralizing antibodies (JE $PRNT_{50}$) using Beijing P3 strain was measured at the Center for Vaccine Development, Mahidol University, in sera samples collected on Day 0, 1 month after the 2nd vaccination, 1 year, and 1 month after the 3rd vaccination. Adverse events were observed for 28 days after each vaccination and serious adverse events were monitored throughout the study. There were 152 enrolled subjects, 79 were male and 73 were female. The mean age was 14.4 months (SD 3.8 months). On Day 0, 5 subjects (3%) had detectable neutralizing antibody levels over the seroprotective level (> 1/10 dilution). One month after the 2nd vaccination, all subjects (100%) had anti-JE level higher than the protective level (GMT 150). Seroprotection rate 1 year after the 2nd vaccination and 1 month after the 3rd vaccination were 89.2% (GMT 49.3) and 100% (GMT 621.7), respectively. The local adverse events included tenderness (0.5%), redness (0.5%), ecchymosis (0.2%). Systemic reactions included fever (17.6%), vomiting (8%), poor appetite (5.3%). No vaccine-related serious adverse events were noted. In conclusion, Inactivated chromatographically purified Vero cell-derived JE vaccine is

safe and immunogenic. It resulted in 100% seroprotection and provided high geometric mean titers after the 2rd and 3rd doses of vaccination. The seroprotection rate after 2 doses at 1 year was also high (89.2%).

1102

AN OVERLOOKED METHOD OF DIAGNOSIS: TROPICAL VIRUSES ISOLATED FROM PHARYNGEAL SWABS

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Tropical viruses, such as dengue virus (DENV), Venezuelan equine encephalitis virus (VEEV), Group C viruses, and hantaviruses cause febrile disease in Peru. Their diagnosis traditionally relies on assays_for example, isolation, PCR, or serology_performed on blood samples. Only rare case reports describe the identification of DENV and VEEV in respiratory samples, and this has never been reported for Group C viruses. As part of an infectious disease surveillance network performed in collaboration with the Ministry of Health of Peru, we collected serum samples from patients presenting with acute febrile disease and tested them using culture or RT-PCR. In addition, in those presenting with respiratory symptoms, an oropharyngeal swab was obtained and initially evaluated for the presence of influenza virus. In order to investigate the sensitivity of pharyngeal swabs in detecting tropical viruses, we retrospectively tested swabs collected from patients with established infection based on their serum sample. Viral isolation and/or RT-PCR was performed on pharyngeal swabs from the following number of patients with established viral infections: DENV, 117; VEEV, 4; and Group C viruses, 1. Virus was detected by viral isolation in 28 (24%), 4 (100%), and 1 (100%) for DENV, VEEV, and Group C viruses, respectively, while PCR detected DENV in 27/77 (35%) and VEE in 4/4 (100%). Also, we identified Rio Mamore virus, a hantavirus previously not associated with human disease, using PCR performed on a pharyngeal swab specimen obtained from a febrile patient. Although potentially not as sensitive as blood specimens, pharyngeal swabs may provide a useful alternative sample collection method to obtain specimens in populations (such as children) that are difficult to obtain blood specimens from.

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GENOMIC AND PHYLOGENETIC CHARACTERIZATION OF BRAZILIAN YELLOW FEVER VIRUS STRAINS

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Globally, yellow fever virus infects nearly 200,000 people leading to 30,000 deaths annually. Although the virus is endemic to Latin America, only a single genome from this region has been sequenced. Here we report 12 Brazilian yellow fever virus complete genomes, their genetic traits, phylogenetic characterization, and phylogeographic dynamics. Variable 3' non-coding region (NCR) patterns and specific mutations throughout the open reading frame altered predicted secondary structures. Our findings suggest that whereas the introduction of yellow fever virus in Brazil led to genotype I a predominant dispersal throughout South and Central Americas, genotype II remained confined to Bolivia, Peru and the western Brazilian Amazon.

1104

BAYESIAN PHYLOGEOGRAPHIC RECONSTRUCTION USING AFRICAN YELLOW FEVER VIRUS ISOLATES INDICATES RECENT SIMULTANEOUS DISPERSAL OF EAST AND WEST LINEAGES

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Yellow fever virus (YFV) is a mosquito-borne flavivirus that is a major public health problem in tropical areas of Africa and South America. There have been detailed studies on YFV ecology in West Africa and South America but current understanding of YFV circulation on the African continent is incomplete. This inadequacy is especially notable for East and Central Africa, for which the unpredictability of human outbreaks is compounded by limitations in both historical and present surveillance efforts. Sparse availability of nucleotide sequence data makes it difficult to investigate the dispersal of YFV in these regions of the continent. To remedy this, we constructed Bayesian phylogenetic and geographic analyses utilizing 49 partial genomic sequences to infer the structure of YFV divergence across the known range of the virus on the African continent. Relaxed clock analysis demonstrated evidence for simultaneous divergence of YFV into east and west lineages, a finding that differs from previous hypotheses of YFV dispersal from reservoirs located on edges of the endemic range. Using discrete and continuous geographic diffusion models, we provide detailed structure of recent African YFV lineage diversity. Significant transition links between extant East and West African lineages are presented, implying connection between areas of known sylvatic cycling. Multiple demographic models reinforce the existence of a stably maintained population of YFV with spillover events into human populations occurring periodically. The layered modeling approach used in the study demonstrates the recovery of ecologically and historically significant structures in the dataset. Presented results justify further incorporation of Bayesian phylogeography into GIS analyses as an augmentation to the study YFV ecology and human disease risk.

1105

PROGRESS TOWARDS THE CONSTRUCTION AND APPLICATION OF MODOC/WEST NILE AND MODOC/CULEX CHIMERIC VIRUSES FOR THE IDENTIFICATION OF GENETIC ELEMENTS THAT MODULATE FLAVIVIRUS HOST RANGE

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Most known flaviviruses, including West Nile virus (WNV), are maintained in natural transmission cycles between hematophagous arthropods and vertebrate hosts. In contrast, other flaviviruses such as Modoc virus (MODV) and Culex flavivirus (CxFV) have host ranges restricted to vertebrates and insects, respectively. The genetic elements that condition these differential host ranges and transmission cycles have not been identified. To address this issue, we are developing chimeric viruses between MODV and WNV as well as MODV and CxFV. Briefly, studies are underway to replace the capsid, pre-membrane and envelope (C-E) genes as well as the pre-membrane and envelope (prM-E) genes of a full-length MODV infectious clone with the corresponding regions of WNV and CxFV. Fusion-PCR will be used to construct junctions between the 3'UTR of MODV and capsid genes of WNV and CxFV as well as the junctions between the envelope genes of WNV and CxFV and the adjacent NS1 gene of MODV. The *in vitro* growth properties of the MODV/WNV and MODV/CxFV chimeras will be compared to those of the parental viruses, and these data will be used to design additional chimeras that contain other genetic regions of the heterogeneous viruses. Overall, these chimeric viruses will provide useful tools for the identification of the genetic determinants that modulate the flaviviral host range.

1106

WEST NILE IN BULGARIA LABORATORY CONFIRMED CASES

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West Nile virus (WNV) is a mosquito-transmitted arbovirus belonging to the genus *Flavivirus* in the family *Flaviviridae*. WNV has a wide geographical range, including Europe, Asia, Africa, and Australia. WNV first appeared in the U.S. in 1999 in New York City. Recently a WNV outbreak occurred in Romania affecting 57 human cases. Samples from acute febrile illness cases (AFI; n=400) were collected from different hospitals in Bulgaria. Clinical and epidemiologic findings, as well as acute and convalescent blood samples were collected from all patients. Samples were tested for WNV among a panel of other potential AFI viruses using IgM and IgG ELISA (Focus Diagnostics, Cypress, CA, USA). Positive WNV IgM cases were further confirmed using indirect fluorescent antibody (IFA; Euroimmune, Germany), PRNT; according to CDC guidelines and rRT-PCR. Two patients had anti-WNV IgM, as determined by ELISA and IFA, and their PRNT was positive at a dilution of 1:20 or more. Both patients had fever and headache; one patient, living in a village bordering Romania, also exhibited neurologic manifestations during the WNV outbreak in 2010. West Nile virus is circulating in Bulgaria. The clinical presentations for these patients were consistent with case reports previously described. A regular surveillance plan of the mosquito population in Bulgaria should be implemented as an early warning system. Further studies are needed to determine the strain of the circulating WNV. This represents the first laboratory confirmed cases of West Nile virus infection in Bulgaria.

1107

EVOLUTIONARY DYNAMICS OF WEST NILE VIRUS IN THE US: DIFFERENTIAL ANALYSIS OF THE PHYLOGENY AND SELECTION PRESSURE IN HUMANS, BIRD AND MOSQUITO HOSTS

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West Nile virus (WNV) is a mosquito-borne virus that is maintained in a bird-mosquito enzootic cycle, but it can also infect other vertebrates including humans. Phylogenetic analyses identified two major WNV genetic lineages: lineage 1 (composed of clades 1a, 1b and 1c) and lineage 2. Recently 3 additional lineages have been described. WNV was first reported in the US in 1999. In 2001, a new WNV genotype (WN02) appeared in the US, displacing the ancestral genotype NY99 by 2003. The WN02 genotype became dominant in the US due to its ability to disseminate more efficiently in domestic mosquitoes as compared to genotype NY99. To date, all strains circulating in the US belong to Clade 1a of lineage 1. In this study we performed evolutionary analyses in WNV isolates circulating in the US. We examined the genetic variation in the open-reading frame (ORF) of 29 selected WNV strains obtained from blood donors during the US epidemics from 2006-2011 and all sequences available in the GenBank (1999-2009) from bird and mosquito-origin. We used maximum-likelihood and Bayesian approaches for phylogenetic analyses and HyPhy for selection pressure analyses. Besides identifying the two main WNV genotypes present in the US (NY99 and WN02), phylogenetic analysis shows that the latter is sub-divided into three groups (g1, g2 and g3). A new sub-type within g3 emerged around 2003 in Southwestern US (SW/WN03). Within this sub-type, we identified a cluster with strains derived from blood donors and birds from the states of ID and ND detected during 2006-2007 (NW/WN06). Few nucleotide and amino acid changes are responsible for the emergence of new US sub-types. We detected a number of codons subjected to positive pressure in structural and non-structural protein genes. Viral adaptation through fixation of spontaneous mutations is an important factor potentially associated with reoccurrence of WNV outbreaks in the New World. The emergence of new genetic variants of WNV raise issues of public health importance because they may affect the sensitivity of both screening and diagnostic assays, as well as the development of vaccines and drugs.

1108

PRODUCTION OF NON-PING PONG DEPENDENT PIWI RNA-LIKE SMALL RNAS IN THE MOSQUITO MIDGUT IN RESPONSE TO WEST NILE VIRUS INFECTION

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Small RNA regulatory pathways are an integral component of endogenous transcriptional regulation, as well as innate immunity. The role of small-interfering RNAs (siRNAs, 20-23 nts) in response to viral infection in invertebrates has been extensively studied and well-characterized. However, the extent to which other small RNA populations participate in antiviral defense is comparably less understood. Recent evidence has suggested that components of the PIWI class of nucleic acid binding proteins can also participate in antiviral defense in mosquitoes. The hypothesis for this observation is that the PIWI-interacting RNA (piRNA) pathway may act as a compensatory response to viral infection when the RNAi pathway is deficient or overburdened. Primary piRNAs show significant strand bias, and range from 24-30 nts in length. Recent studies with Chikungunya virus and Sindbis virus (*Togaviridae*) in *Aedes aegypti* and *Ae. albopictus* mosquitoes and cell lines have described the production of viral-derived piRNA-like small RNAs that exhibit "ping-pong" amplification. In this model, primary piRNA transcripts with a strong bias for a 5′ uridine terminus bind target transcripts and result in cleavage of the target 10 nucleotides upstream from the 5′ uridine residue. Due to this, secondary piRNAs produced in this manner exhibit an adenine residue in the 10 position. Here we characterize 24-30 nt piRNA-like small RNAs produced in response to West Nile virus (*Flaviviridae*) infection in *Culex quinquefasciatus* mosquitoes. Interestingly, while exhibiting a strong bias for sense-strand orientation, viral-derived piRNA-like RNAs did not exhibit signatures indicative of ping-pong amplification. Previous studies of dengue virus-infected *Ae. aegypti* support this observation, suggesting that the piRNA pathway may function differently in response to flaviviruses compared to alphaviruses.

1109

URBAN-RURAL DIFFERENCES IN THE IMMUNE GENE EXPRESSION PROFILE OF GHANAIAN CHILDREN

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Urbanization is having dramatic effects on disease patterns in developing countries. However, little is known about corresponding immune system changes associated with this process. A key mechanism underlying immune response is variation in gene expression which is controlled by genetic as well as environmental factors. Exploring immune gene expression patterns between urban and rural populations could provide insights into the impact of urbanization on changes in the immune profile as well as on disease outcomes. Our aim was to determine whether different geographical environments in one region of Ghana impacted on mRNA expression levels of selected immune genes. Our study population was 151 children aged 5-13 years attending rural, urban low socioeconomic status (SES) and urban high SES schools in the Greater Accra Region of Southern Ghana. Parasitological samples were collected to detect helminth infections and malaria parasites. Gene expression was examined in *ex vivo* whole blood samples using real-time quantitative

PCR. Selected polymorphisms of the IL-10 gene as well as selected polymorphisms of Toll-like Receptor (TLR) genes were also genotyped using the MassARRAY system. *S. haeamatobium* infection was detected among rural but not urban children. Intestinal helminths were found in all three areas but were highest among rural subjects followed by the urban low SES school and lastly the urban high SES school. Malaria was prevalent in the rural area and negligible in the urban area. Marked differences in gene expression were observed between the rural and urban areas as well as within-urban variations based on socioeconomic level. Current *S. haeamatobium* infection modulated the expression of some genes involved in the TLR signalling pathway and accounted for the urban-rural differences found with respect to these genes. Interestingly, IL-10 gene expression was elevated in the rural compared to urban area ($p<0.001$) but this was not associated with current helminth infection. In addition, elevated IL10 mRNA levels were influenced by genetic polymorphisms in the IL10 gene but these did not explain the urban-rural differences observed. In summary, we ascertained that immune gene expression patterns are strongly influenced by environmental determinants and can serve as important markers of the effects of urbanization on immune profile changes within the tropics.

1110

THE *LOA LOA* GENOME AND ITS IMPLICATIONS FOR THE *WOLBACHIA*-FILARIA SYMBIOSIS

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Loa loa, the African eyeworm, is the least well-studied of the human pathogenic filarial parasites but is gaining clinical prominence because of serious adverse events following antifilarial treatment. Furthermore, unlike any of the other filarial parasites of humans, *L. loa* does not contain the alpha-proteobacterial *Wolbachia*, considered to be an obligate endosymbiont of most filarial worms. Therefore we generated a 20x draft genome sequence of *L. loa*, and a 12x draft genome of the related filarial parasite *Wuchereria bancrofti* for comparative purposes. The *L. loa* genome assembly is 91.4 Mb with a scaffold N50 of 172 Kb, making it the most contiguous filarial genome assembly to date. RNA-Seq data generated from *L. loa* microfilariae was used to generate a high-quality annotation of the *L. loa* transcriptome, which is predicted to encode 14,925 genes. Gene order is highly conserved between *L. loa* and the related parasites *W. bancrofti* and *Brugia malayi*. Using orthology to *C. elegans*, we profiled the metabolic biosynthesis and transport capabilities of the three filarial worms, in addition to five other free-living and parasitic nematodes. All nematodes showed remarkable conservation of metabolic pathways, with the exception of purine biosynthesis which has been lost independently in multiple lineages. Despite lacking intracellular *Wolbachia*, *L. loa* shows no new metabolic synthesis or transport capabilities relative to the other filarial parasites, and no evidence of extensive lateral gene transfer from *Wolbachia* or from other bacteria. These results suggest the metabolic role of *Wolbachia* symbionts is likely more subtle in nature than providing otherwise unobtainable nutrients.

1111

IMPROVED QPCR ASSAYS FOR DETECTING FILARIAL DNA IN BLOOD OR MOSQUITOES

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Molecular detection of filarial DNA can be used for mapping endemic areas and for monitoring elimination programs. We previously reported highly sensitive TaqMan qPCR assays for detecting *Wuchereria bancrofti* (Wb) and *Brugia malayi* (Bm) DNA in blood and mosquitoes. The current study compared the performance of qPCR with two different master mixes, namely TaqMan (Reagent A) and SsoFast (Reagent B). Reagent B contains a unique Sso7d fusion polymerase that increases the efficiency and speed of PCR reactions compared to the Taq polymerase present in the TaqMan mastermix. qPCR assays for Wb, *Dirofilaria immitis (Di), D. repens* (Dr) and Bm DNA employed primers and TaqMan probes specific for LDR, MTR1, MTR2, and HhaI- repeat DNA sequences, respectively. The efficiencies of all of these qPCR assays with Reagent A or B were close to 100%. The analytical sensitivity for detecting DNA was the same with either assay for Wb and Di, but the Reagent B assay was more sensitive for Dr and Bm DNA. The detection limits for Wb LDR plasmid DNA and for Di, Dr and Bm genomic DNA were approximately 0.01fg, 100fg,1fg, and 1fg, respectively. Cycle threshold values with reagent B were 2-10 cycles lower (4 to 1000 fold more sensitive) than with Reagent A. qPCR assays for Dr and Bm DNA with Reagent B were more sensitive than assays for Wb and Di DNA with either Reagent A or B. All qPCR assays with Reagent A or B master mixes were species-specific, with no signals detected with DNA templates from other filarial nematodes, *Plasmodium falciparum*, *Aedes aegypti*, or *Homo sapiens*. We compared the performance of the two Wb qPCR assays with field samples from a Wb endemic area. Results were the same with both assays for 50 pools of gravid mosquitoes collected in Wb-endemic areas (24 positive pools) and for 50 human blood samples (17 positives). 15 of 15 dog blood samples with Dr microfilariae were also positive by qPCR with both mastermixes, and no false positive results were observed with uninfected dog blood samples. Additional studies are needed to evaluate the Reagent B qPCR assays with other field samples (blood and mosquitoes). Reagent B costs much less than Reagent A, and this impacts the total cost per PCR reaction. Thus Reagent B qPCR assays provide significant advantages for probe-based qPCR assays for detecting filarial DNA in blood samples and in mosquitoes.

1112

UNRAVELLING THE MUTUALISTIC SYMBIOSIS OF *WOLBACHIA* AND THE FILARIAL NEMATODE *BRUGIA MALAYI*: A SYSTEMS BIOLOGY APPROACH

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The parasitic nematode *Brugia malayi* is a causative agent of lymphatic filariasis, a disfiguring disease affecting over 120 million people worldwide. *B. malayi* exists in a mutualistic symbiotic relationship with *Wolbachia*. Larval development, embryogenesis and adult worm survival are the key biological processes, entirely dependent on this symbiosis. We have applied an omics approach to investigate the molecular basis of this symbiosis. In order to study the role in larval and embryonic development, Illumina RNA Seq was used to produce a comprehensive transcriptome of four time points spanning the early post-infection development of *B. malayi* in the mammalian host *Meriones unguiculatus* and at multiple time-points post-antibiotic depletion from adult females. *Wolbachia*/worm ratios within developing larvae and adult worms at each time point were also monitored by qPCR and fluorescent microscopy. In parallel proteomic profiling of these selected nematode life cycle stages has been adopted to monitor protein expression of *Wolbachia* and *B. malayi*. In-solution tryptic proteolysis coupled with reversed phase liquid chromatography and analysis by high-resolution mass spectrometry provides a powerful tool for global proteome analysis. This initial shot-gun approach has been optimised to include an extensive pre-fractionation step to delve deeper into the proteome by increasing peptide and protein identification. Following basic analysis through established bioinformatics pipelines, transcriptomic, proteomic, and published datasets will be integrated in a systems biology approach with the objective of understanding the molecular basis of the mutualistic *Wolbachia*/*B. malayi* symbiosis.

1113

DEMONSTRATION MIRNA-MEDIATED REGULATION OF EXPRESSION WITH TRANSIENTLY TRANSFECTED *BRUGIA MALAYI* EMBRYOS

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miRNAs have been implicated in transcriptional, post-transcriptional and translational regulation in gene expression. Recently, deep sequencing experiments have demonstrated the presence of miRNAs in parasitic nematodes, including *Brugia malayi* and *Ascaris suum*. However, little is known concerning the role that these miRNAs play in regulating gene expression in these organisms, or the mechanisms through which they might exert their effects. As a first step in exploring the role of miRNAs in *B. malayi*, the target sequence for the *B. malayi* mir-71 miRNA was inserted into the 3′ untranslated domain of a reporter construct consisting of the *B. malayi* HSP70 promoter driving the expression of a luciferase reporter gene containing the first intron of the native BmHSP70 gene and the BmHSP70 3′ UTR. mir-71 was chosen for this study because deep sequencing revealed that it was a very abundant endogenous miRNA. Insertion of the mir-71 target sequence resulted in a decrease in reporter luciferase activity in embryos transfected with this construct to 20% of the level seen in embryos transfected with the un-mutated construct. Mutation of the 3′ end of the mir-71 target (corresponding to the putative mir-71 seed sequence domain) restored the reporter activity to 80% of that seen in with the wild type construct. Similarly, mutation of the 5′ end of the target site resulted in reporter activity which was 50% of that seen with the wild type. In contrast, mutation of the putative slicer recognition site of the mir-71 target sequence did not result in any restoration of activity. These data suggest that the mir-71 miRNA is capable of interacting with and reducing protein expression from mRNAs containing its target sequence. This study further demonstrates that transient transfection of *B. malayi* with constructs containing reporter genes can be used to explore the function of miRNAs in the human filarial parasites.

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NOVEL INHIBITORS OF THE *BRUGIA MALAYI* STRESS-ACTIVATED PROTEIN KINASE, BM-MPK1

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Filariasis, caused by thread-like nematode worms, affects millions of individuals throughout the tropics and is a major cause of acute and chronic morbidity. Filarial nematodes effectively evade host immunological responses and are long lived within their hosts. In particular, filarial parasites are particularly resistant to oxidative stress generated by reactive oxygen species (ROS) generated during infection by cells of the innate immune system. We have previously characterized a stress-activated protein kinase, Bm-MPK1, from the filarial parasite *Brugia malayi*, which is related to the evolutionarily conserved human mitogen-activated p38 protein kinase (p38) and the *Caenorhabditis elegans* PMK-1 protein kinases. We have demonstrated inhibition of Bm-MPK1 activity using a panel of known p38 inhibitors. Furthermore, treatment of *B. malayi* adult and larval forms with p38 inhibitors in the presence of ROS compromises the ability of the parasite to effectively mount an anti-stress response leading to the death of the parasite. Inhibition of this pathway may have therapeutic benefit in treating filariasis by increasing the sensitivity of filarial parasites to ROS and other reactive intermediates. We now report on the results of a Bm-MPK1 kinase screen using a focused kinase library of p38 inhibitors. The initial screening plate was comprised of 40 compounds covering 8 structural series. After two follow-up rounds of screening, covering an additional 74 compounds in 3 series, a lead series was selected. Several inhibitors were identified exhibiting potencies ranging from 14-116 nM and exhibiting a 2-5-fold selectivity for Bm-MPK1 over human p38. Several compounds were effective in blocking parasite responses to ROS. Two compounds in particular, Compound 001 & 002, effectively induced parasite death in the presence of ROS at concentrations of ~ 5uM. These results indicate the potential for development of Bm-MPK1 selective inhibitors as anti-filarial therapeutics.

1115

ANTI-*WOLBACHIA* CONSORTIUM (A·WOL) DRUG DISCOVERY: SCREENING DIVERSITY LIBRARIES TO DISCOVER NOVEL AREAS OF CHEMICAL SPACE WITH ANTI-*WOLBACHIA* PROPERTIES

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There is an urgent need to develop new drugs for onchocerciasis and lymphatic filariasis treatment and control. The Anti-Wolbachia Consortium (A·WOL) is testing a diverse range of compounds to find new chemical space to meet this demand. 558,000 compounds have been procured from the following libraries: Medicines for Malaria Venture (MMV - 500,000 compounds), BioFocus® (10,000 compounds), and Shanghai Institute of Materia Medica (SIMM - 48,000 compounds). 12,400 of these compounds have already been screened with 130 hits identified as reducing *Wolbachia* levels by >90%. Hits are scrutinised to assess suitability for further assessment of structure-activity relationships and select the best candidates to take forward as part of the drug discovery program. We are exploring a range of cheminformatic approaches to allow us to rapidly identify groups of compounds that show anti-*Wolbachia* activity and reject those where the chemical space is largely redundant.

1116

EVALUATION OF IGG4 IMMUNE RESPONSES AGAINST OV-16 AND BM-14 IN NON-HUMAN PRIMATES INFECTED WITH *ONCHOCERCA VOLVULUS*

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We studied the temporal evolution of immune responses from six chimpanzees inoculated with 200-400 L3s from *Onchocerca volvulus*. Serum samples and skin snips were systematically collected on a monthly basis for 4-5 years post inoculation. Patent infections were confirmed by detection of microfilaria (Mf) in skin snips. Serological IgG4 responses were evaluated by ELISA against recombinant antigens OV-16 (onchocerciasis) and Bm-14 ((lymphatic filariasis [LF]). Mf were detected at ≥11 months post-inoculation (mean=485 days, median=406, range 336-763 days). Positive immune responses against Ov16-IgG4 were observed before Mf were detected (mean=453, median 462 days), There were no significant differences between detection of Mf and OV-16 seroconversion (p=0.53, paired t-test) showing that both methods have similar temporal diagnostic value. Anti-Bm14-IgG4 responses were always detected after Mf positivity (mean 670 days, median 601), however these differences were not statistically significant (p=0.18). The cross-reactivity with Bm14 is important in areas where onchocerciasis and LF are co-endemic. Our findings suggest that serological evaluations may need an additional highly specific LF antigen to discriminate between onchocerciasis and LF. Further

population-based studies are required to confirm the reactivity of sera from onchocerciasis patients with Bm-14, and the potential use of this antigen as a pan-filarial screening reagent.

1117

FILARICIDAL DRUGS INDUCE APOPTOSIS IN THE FILARIAL NEMATODE-*BRUGIA MALAYI* AND THIS EFFECT IS NOT PRIMARILY DUE TO *WOLBACHIA* DEPLETION

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Filarial nematodes harbor *Wolbachia* bacteria and depletion of *Wolbachia* in filarial nematodes results in defects in nematode development, fertility and viability. Induction of apoptosis in *Brugia malayi* upon *Wolbachia* depletion was shown and thus suggested the involvement of factors originating from *Wolbachia*, as reported previously. To further confirm that apoptosis occurring during anti-filarial drug treatment is caused by *Wolbachia*'s depletion, we compared two types of drugs: a macrofilaricidal drug which doesn't target *Wolbachia* such as flubendazole and gentamycin, an antibiotic known to be ineffective in killing *Wolbachia* vs. tetracycline, which targets *Wolbachia*. Adult *B. malayi* worms were cultured *in vitro* and treated with 40 µg/ml of tetracycline, 20 µg/ml of flubendazole or 40 µg/ml of gentamycin. As a control for effects of death, we used untreated adult worms that were killed by freezing. Tetracycline and flubendazole killed *B. malayi* adult worms on day 5 and day 6 of treatment, respectively where as with gentamycin treated worms survived for 10 days and untreated worms survived for more 2 weeks. Treated worms were harvested at the point of death or alive (untreated worms) and were sectioned and stained for *Wolbachia* and a Tunnel assay was performed to detect apoptosis. Interestingly, minor degree of apoptosis was already found in untreated worms. These untreated worms harbored many *Wolbachia* based on immunhistology and qPCR. In untreated worms killed by freezing also showed signs of apoptosis although *Wolbachia* could be detected in these worms. In tetracycline treated worms, there were extensive signs of apoptosis and there was also a marked reduction in staining for *Wolbachia*, however, worms treated with flubendazole although showed extensive apoptosis numerous *Wolbachia* could be still be detected. Apoptosis was also observed in gentamycin treated worms while numerous *Wolbachia* were still detected. Thus our results show that induction of apoptosis following anti-filarial drug treatment is mainly caused by worm death due to the drug's activity and its toxicity and is not necessarily due to the depletion of *Wolbachia* alone, as proposed earliar.

1118

IN 2012, CARTER CENTER ASSISTED RIVER BLINDNESS PROGRAMS HALTED OVER 1.2 MILLION IVERMECTIN TREATMENTS IN FOUR COUNTRIES AFTER TRANSMISSION INTERRUPTION WAS DEMONSTRATED

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The Carter Center (TCC) assists national ministries of health in 11 countries in Africa and the Americas to conduct health education and distribute the medicine ivermectin (Mectizan®, donated by Merck) for onchocerciasis. Where possible, programs aim to break the transmission of onchocerciasis as well as control the associated morbidity. TCC partners include national governments, WHO/PAHO, Gates Foundation, Lions Clubs, CDC, and several universities. TCC assisted elimination efforts have been based on more intensive use of ivermectin, with twice or even four times per year treatments, and (in Uganda), vector control. Complete elimination of Onchocerca volvulus is the goal in six countries in the Americas, Uganda and Sudan. Four programs (Mexico, Guatemala, Sudan and Uganda) interrupted transmission in five transmission zones in 2011, which has resulted in withdrawal of an estimated 1,235,000 treatments in 2012. These programs have followed the 2001 WHO guidelines for documenting elimination of onchocerciasis as applied by in the Americas by the OEPA and Pan American Health Organization, and by the ministries of health of Uganda and Sudan. The include demonstration that OV16 antibody tests are < 0.1% positive in children, and PCR assays in vectors demonstrate fewer than 1/2000 infective black flies. In African programs, TDR guidelines are also included where skin snip surveys must show microfilaria (mf) prevalence are less than 5% in all sampled communities (and less than 1% in 90% of sampled communities). TCC assisted transmission zones where treatment is being withdrawn will now enter three years of post treatment surveillance (PTS).

1119

ROLE OF INDUCIBLE NITRIC OXIDE SYNTHASE (INOS) IN MICE WITH EOSINOPHILIC MENINGITIS CAUSED BY *ANGIOSTRONGYLUS CANTONENSIS* INFECTION

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Nitric oxide (NO) levels in the CSF was found to be a useful prognostic marker in bacterial and tuberculous meningitis, but few studies were conducted in eosinophilic meningitis. Inducible nitric oxide synthase(iNOS) was found to have a detrimental role in the pathology resulting from acute cerebral injury. Inducible NOS was also found to mediate hippocampal caspase-3 activation in pneumococcus meningitis. However, iNOS knockout mice infected with M. tuberculosis developed serious clinical manifestations and granulomatous lesions containing tubercle bacilli throughout the meninges, all of which were absent in wild-type mice. This indicated that importance of NO in defense against TB meningitis. In this study, we used the inducible nitric oxide synthase (iNOS) knockout mice to analyze the brain pathological and apoptotic changes in mice with eosinophilic meningitis. Wild type and iNOS knockout mice were orally infected with 50 *A. cantonensis* L3 via an orogastric tube after slight ether anesthesia and then 6 mice were sacrificed every week for 4 consecutive weeks after infection until the end of the study. Serial brain protein homogenates were used for analyzing the apoptotic protein changes by western blot analysis. Hematoxylin-Eosin staining of brain and TUNEL assay were done for comparing the pathologic changes in wild type and knockout mice. It was found that the iNOS knockout mice had more severe cerebellar inflammation as evidence by the more severity of eosinophilic meningitis, granulomatous encephalitis and perivascular cuffing in cerebellum. By using TUNEL and western blot analysis, it was found that the iNOS knockout mice had more severe apoptosis changes in cerebellum compared to the wild type mice following different weeks of infection. In conclusion, we found that the iNOS is probably protective against parasitic associated eosinophilic meningitis.

1120

THE IMPACT OF A SCHOOL-BASED HYGIENE, WATER QUALITY AND SANITATION INTERVENTION ON SOIL-TRANSMITTED HELMINTH REINFECTION: A CLUSTER-RANDOMIZED TRIAL

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Periodic chemotherapy is a cost-effective approach to reducing soil-transmitted helminth infection. However, without exposure mitigation, reinfection can occur rapidly. We conducted a cluster-randomized trial to assess the impact of a school-based hygiene promotion and sanitation program on reducing infection with soil-transmitted helminths (STH) following a school deworming campaign. Reinfection with soil-transmitted helminths was quantified twice over two years following a baseline measurement and deworming. Forty government primary schools from three administrative districts in Nyanza Province, Kenya were randomly selected and assigned to intervention or control arms. The intervention included latrine construction, provision of handwashing stations, and hygiene education. A random selection of 2,904 school pupils at three time points were assessed for prevalence and intensity of STH infection using stool samples. Observations were conducted at the pupils' homes to assess household water, sanitation, and hygiene conditions and socio-economic status. The impact of the intervention on the prevalence of *Ascaris lumbricoides* was found to be significant for girls (Odds Ratio [OR] 0.49, 95% confidence interval [CI] 0.25-0.98), but not for boys (OR 0.98, 95% CI 0.52-1.88); the effect on intensity of infection followed a similar pattern. There were no significant effects of the intervention on the prevalence and intensity of *Trichuris trichiura* or on the prevalence of hookworm. For the intensity of hookworm infection, stratification by gender revealed a significant impact among boys (IRR 0.21, CI 0.08-0.57) and a trended, though non-significant increase on girls (IRR 2.12, CI 0.86-5.20). Children with lower levels of water, sanitation, and hygiene access at home benefitted more from the school-based program. Provision of school-based sanitation, water quality, and hygiene improvements may reduce reinfection of soil-transmitted helminths following broad-based deworming. The magnitude of the effects may be gender- and worm-specific, pointing to behavioral characteristics associated with reinfection. That poorer children and those with lower access to improved WASH benefitted more from the program has important implications for the equity of WASH provision and health improvement.

1121

EVIDENCE THAT MULTISECTOR FOOD SECURITY INTERVENTION PROGRAM IN RURAL PANAMA REDUCES HOOKWORM INFECTION IN PRESCHOOL CHILDREN

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Since 2007, a multisector food security program in rural Panamanian communities has attempted to improve food security and health through agriculture extension, training in maternal-child nutrition and hygiene, and community organisation. Although gastrointestinal parasites are recognized as a health problem among young children, they have been outside the scope of the program. Our objective was to compare prevalence and intensity of intestinal parasitic infection among preschool children (6-60 mo) not yet involved in the program and those involved for 1 or 5 years. Fecal samples from 153 children were examined for Protoza (direct smear) and helminths (FLOTAC), and household demographics, hygiene behaviours, and sanitation and water infrastructure were noted. An improved household water source was found in 43% of households and was more common where the program had begun in 2007 ($p = 0.0003$). Latrines were found in 82% of homes and 59% of caregivers reported that their child always wore shoes. Hookworm was found in 31% of children, *Giardia* in 28% and *Ascaris* in 16%. Stepwise multiple regression models revealed that hookworm epg was higher in older children, households with more preschool children, and households without an improved water source ($p = 0.0001$). *Ascaris* epg was higher in children with younger caregivers and in households with more children 12 years and younger ($p = 0.02$). Presence of *Giardia* was not correlated with any of the measured demographic, behaviour or infrastructure variables. The number of years involved with the program did not emerge as a factor contributing to either prevalence or intensity of any of the infections. Our results show that that improved water source is associated with reduced hookworm infection but that long-term participation in this multisectoral program alone is not sufficient to protect against gastrointestinal parasites. Our future work will explore whether specific aspects of this multisector intervention are effective in reducing transmission of gastrointestinal parasite infections.

1122

OXANTEL PAMOATE: REVIVAL OF AN OLD DRUG

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Recent estimates suggest that 4.5 billion people are at risk of soil-transmitted helminthiasis (STH) with over 1 billion people currently infected with one or more of the three common soil-transmitted helminths (i.e. *Ascaris lumbricoides*, hookworm and *Trichuris trichiura*). The global strategy for the control of STH is preventive chemotherapy, however, only a handful of drugs are available against STH, i.e. albendazole, mebendazole, levamisole and pyrantel pamoate). It is recognized that none of the drugs are efficacious against all three STH species with particularly low cure rates observed against *T. trichiura*. While, individually, compounds may not meet the desired target product profile, combination of drugs with different characteristics may achieve higher levels of efficacy then the individual products, which might prolong the useful lifespan of the existing drugs by conferring mutual protection against resistance. We studied the trichuricidal potential of the "old", veterinary drug oxantel pamoate and the effect of oxantel pamoate combined with albendazole, mebendazole, levamisole, pyrantel pamoate and ivermectin *in vitro* and *in vivo*. We calculated an ED_{50} of 4.7 mg/kg for oxantel pamoate against Trichuris muris in mice. For comparison, albendazole, mebendazole, levamisole and pyrantel pamoate are characterized by ED_{50} values of 345 mg/kg, 79 mg/kg, 46 mg/kg and > 300 mg/kg. Oxantel pamoate combined with levamisole and combinations of oxantel pamoate with pyrantel pamoate behaved antagonistically. Highly synergistic effects (combination index <1) were observed when oxantel pamoate-mebendazole was administered *T. muris* infected mice. For the combination oxantel pamoate-albendazole a nearly additive behavior was determined *in vivo*. In conclusion, our study confirmed the excellent trichuricidal properties of oxantel pamoate. Further preclinical studies are warranted with the two lead candidate combinations oxantel pamoate-mebendazole and oxantel pamoate-albendazole.

1123

TRENDS IN INTESTINAL PARASITISM IN A MILITARY POPULATION IN THE PERUVIAN AMAZON, 2003-2011

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In the Amazon Basin, intestinal parasitism is endemic and is a major cause of morbidity in military and civilian populations. We investigated the trends in intestinal parasitism in a military population operating in the Peruvian Amazon to help guide diarrheal disease treatment and deworming efforts. From 2003 to 2011, all asymptomatic individuals newly stationed at the

Vargas Guerra Army Base in Iquitos were invited to provide a stool sample upon enrollment in a longitudinal study of diarrheal disease. At least one species of intestinal parasite was identified in 74% (3284/4406) of stool samples, and 50% (1650/3284) of infected individuals tested positive for multiple parasitic species. The most prevalent parasites identified were *Ascaris lumbricoides* (40%), *Entamoeba coli* (35%), *Trichuris trichura* (19%), *Uncinaria stenocephala* (16%), and *Giardia lamblia* (16%). The prevalence of intestinal parasitism increased from 67% in 2003 to 81% in 2011 (Odds Ratio (OR)=1.23, 95% CI= 1.19, 1.28; p<0.001), and the probability of multiparasitism among parasite-infected individuals also increased over time (OR=1.15, 95% CI=1.11, 1.19; p<0.001). These trends persisted even after adjustment for age, living quarters, and provenance. These changes in the prevalence of parasitic infection appear to be driven by significant increases in the prevalence of *A. lumbricoides* (OR=1.03, 95% CI=1.00, 1.06), *T. trichura* (OR=1.14, 95% CI=1.10, 1.18), and *G. lamblia* (OR=1.07, 95% CI=1.03, 1.11), as the prevalence of all other parasites tested either decreased or remained stable over the study period. These data demonstrate the high prevalence of intestinal parasitic infection in the Peruvian Amazon and the need for further investigation of their impact on troop readiness in the region.

1124

COMPARISON OF THE FECAL PARASITE CONCENTRATOR METHOD TO KATO-KATZ FOR THE DIAGNOSIS OF SOIL TRANSMITTED HELMINTHS

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The World Health Organization (WHO) recommends that the Kato-Katz (KK) method be used to diagnose soil transmitted helminth (STH; *Ascaris sp.*, *Trichuris sp.*, and hookworm) infection. However, stool samples analyzed using KK must be evaluated within 24 hours after the sample is produced, creating difficulties during field studies. In settings where laboratory diagnosis is not readily available, alternative techniques that allow stool preservation for transport and later examination are needed. The objective of this study was to compare the sensitivity of the KK method with a formalin preservation technique known as the Mini Parasep® Fecal Parasite Concentrator (FPC) method. In the absence of an accepted "gold standard" test, we will use latent class analysis to compare the results of each test in identifying *Ascaris sp.*, *Trichuris sp.*, and hookworm infections. In 2010, we collected stool samples from 324 residents ≥ 1 year old in randomly selected households in the Guatemalan county of Nueva Santa Rosa. Part of each sample was preserved in formalin in the field using a pre-filled Mini Parasep® Fecal Parasite Concentrator container and the remainder was left unpreserved. Samples were stored in a cooler and transported to the lab during one of two trips each day, and then tested by both methods. KK testing was performed following the WHO recommended procedure, and FPC using the Mini Parasep® protocol. Seventeen (5.3%) individuals tested positive for *Ascaris sp.* by FPC, while 28 (8.6%) tested positive by KK. Three (0.9%) individuals tested positive for *Trichuris sp.* by FPC, while 16 (4.9%) tested positive by KK. One person (0.3%) tested positive for hookworm, which was found by FPC but not KK. Latent class analysis will be utilized to further compare the results of the FPC and KK methods. Although the KK method poses logistic challenges for field surveys, the alternative formalin-preservation FPC method is less sensitive compared to the KK method for the 3 pathogens tested. New diagnostic tools for STH that combine the ease of the FPC method and the sensitivity of the KK method are needed.

1125

HEALTH-SEEKING BEHAVIORS AND TREATMENT FOR SOIL TRANSMITTED HELMINTH INFECTION IN NUEVA SANTA ROSA, GUATEMALA - 2010

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Infection with a soil-transmitted helminth (STH) (e.g., *Ascaris sp*. and hookworm) can lead to dysentery, anemia, and cognitive impairment, especially in children. Annual or bi-annual treatment with antihelminthic drugs has been shown to reduce infection intensity in affected individuals. To prevent STH-associated morbidity and mortality, the Guatemalan Ministry of Public Health and Social Welfare conducts a semi-annual school-based mass drug administration (MDA) for STH throughout the country. This study examines STH treatment practices and health-seeking behaviors for STH infection in the Guatemalan county of Nueva Santa Rosa (NSR). We collected stool samples from 324 residents ≥ 1 year of age in randomly selected households in NSR between July -August 2010. Samples were tested using both Kato-Katz and Mini Parasep® Fecal Parasite Concentrator methods. Individuals positive for any of the three STHs on either test were considered infected. STH prevalence was 13.3% (N=43/324). STH burden was highest in preschool-aged (PSAC) (7/54, 13.0%) and school-aged children (SAC) (20/104, 19.2%); 9.6% of adults were infected. Overall, 110 (34.1%) individuals reported taking drugs to treat intestinal worms during the previous year; this proportion did not differ significantly between infected and uninfected individuals (p=0.71) though both PSAC and SAC were significantly more likely to receive antihelminthic drugs compared to adults (OR: 4.4, 95% CI: 2.2-8.9; OR: 2.3, 95% CI: 1.3-4.2; respectively). Of those taking drugs, 43.3% (N=45) obtained them at a store or pharmacy, and 37.5% (N=39) from a hospital or clinic. Observing worms in stool in the previous year and regular school attendance were not associated with STH positivity on univariate analysis. Multivariable logistic regression modeling will be used to examine significant risk factors for STH infection in this sample. Treatment with antihelminthic drugs was infrequent in this sample, even among SAC, where only 40% reported receiving treatment in the previous year. These results suggest that school-based anti-helminthic treatments are not reaching a substantial portion of the school-aged population in this part of Guatemala. Given that nearly 10% of adults were infected with a STH and only 23% reported receiving antihelminthic drugs, additional research should evaluate whether individuals age 18 years or older act as reservoirs for STH infection.

1126

COMPONENTS OF AN ANTIGEN CAPTURE ASSAY FOR THE DIAGNOSIS OF *ASCARIS* INFECTION

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Ascaris is the largest soil transmitted helminth known to cause human infection. It is estimated to affect one sixth of the world's population causing malnutrition, poor school performance and decreased response to vaccines. Current diagnosis of ascariasis is made using the Kato Katz method of microscopic examination of stool specimens. This method is time and labor intensive, technician-dependant and is not possible until one month into the infection when the life cycle is complete. Serological assays have been unreliable because of differences in host responses to Ascaris antigens. The development of an assay that could detect Ascaris

antigen in host bodily fluids in the early phase of infection would have wide applicability and utility. Immunoscreening of an A. suum infective larval stage 2 cDNA library was performed using sera from infected swine which identified ABA-1 as the immunodominant antigen at this stage. ABA-1 is an Ascaris antigen that has previously been described as a component of the Ascaris ES (excretory-secretory) protein which is produced and excreted by all stages of the parasite making it an excellent antigen target. ABA-1 protein is expressed by both A. suum which infects swine and A. lumbricoides which infects humans allowing a wider applicability of any successful assay. Recombinant ABA-1 fusion protein was produced from the ABA-1 containing clones using DNA isolated from the immunoscreening process. This DNA segment is homologous to that of the Ascarsis lumbricoides ABA-1. Two monoclonal anti-ABA-1 antibodies were generated by murine immunization and subsequent formation of immune B cell and murine myeloma hybridomas. Anti-ABA-1 activity has been confirmed by both ELISA and western blot assays to the recombinant ABA-1. Further characterization of the antibody epitopes should allow the development of an ELISA capture assay to aid in early diagnosis of Ascaris infections both in swine and humans.

1127

PREVALENCE AND SOCIODEMOGRAPHIC RISK FACTORS OF HELMINTH INFECTIONS AMONG ADULTS IN RURAL SOUTHWESTERN KENYA

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Rural southwestern Kenya suffers from high soil-transmitted helminth (STH) prevalence. Two studies of a rural village in the Nyanza Province demonstrated a pediatric STH prevalence that fell from 68% in 2007 to 44% in 2010, likely due to increased access to clean water, hygiene and sanitation training, and improved deworming coverage in the schools. We hypothesized that adults (≥18 years) have similarly high prevalence of STH infections and serve as reservoirs for re-infection of the children. We determined the prevalence of STH infection in the adults of this village and assessed socio-demographic factors associated with STH prevalence. We collected stool samples of 323 adults recruited at the local hospital and at four local schools. Basic demographic data were collected before stool sample screening. We used a highly sensitive sedimentation concentration technique for each sample. Concentrated specimens were stained with Dobell's iodine and examined for ova and parasites by light microscopy at 40x magnification. Adjusted prevalence ratios (PR) were estimated using multivariable regression. Prevalence of STH was 15.8% (51/323). Hookworm was most common (13.9% prevalence), followed by *Ascaria lumbricoides* (5.6%), and *Trichuris trichiura* (0.6%); no *Strongyloides stercoralis* infections were identified. Twelve participants (3.7%) had multiple STHs. Higher prevalence was associated with female sex (PR = 1.86; 95% CI: 0.97, 3.57) and age over 55 (1.94; 1.09, 3.45). Lower prevalence was associated with completion of some secondary school (0.17; 0.04, 0.71), occupation other than farming (0.30; 0.09, 0.95), and presence of any children living in the household (0.66; 0.34, 1.27). The STH prevalence of 16% found among adults was approximately one-third of that found among children, and may warrant regular de-worming of adults with risk factors for STH infection.

1128

COMPLETE CURE OF HUMAN HOOKWORM IN A HAMSTER MODEL USING A NOVEL APPROACH WITH CRY5B FROM *BACILLUS THURINGIENSIS*

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Two billion people in the world are infected with soil-transmitted helminths. The infections have devastating effects on human growth, nutrition, cognition, school attendance/performance, earnings, and pregnancy. Furthermore, the infections negatively affect HIV, malaria, tuberculosis, and modulate immune response, as reported previously. Currently the WHO recognizes 4 drugs (2 nicotinic acetylcholine receptor agonists, 2 benzimidazoles) for human treatment. The primary drug used for a variety of reasons is albendazole, and resistance to this drug is quickly emerging. Cry5B, a protein naturally produced in *Bacillus thuringiensis*, has been well characterized as an anthelmintic. Other Crystal family member proteins have been shown to be safe in humans, for example in transgenic corn, as a natural pesticide. This safety is thought to stem from multiple factors including the binding to an invertebrate-specific glycolipid receptor. However, there has never been a complete cure using Cry5B for *in vivo* experiments. The best, published result with Crystal protein (Cry5B) was an 89% hookworm reduction, as reported previously. Thus, we developed a novel method for using Cry5B as an anthelmentic. Consequently, we treated hamsters infected with human hookworm (*Ancylostoma ceylanicum*) with either Cry5B or control and achieved 100% cure in the Cry5B treated animals while the control treated animals had ~45 hookworms per intestine. This result constitutes the first time a 100% cure of intestinal worm burden has been achieved with a Crystal protein.

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FIRST-IN-HUMANS CLINICAL TRIAL OF THE *NA*-GST-1 HOOKWORM VACCINE IN BRAZILIAN ADULTS

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Necator americanus glutathione S-transferase-1 (*Na*-GST-1) is a 24-kDa protein that has peroxidase enzymatic activity that catalyzes the conjugation of reduced glutathione to a variety of electrophiles. *Na*-GST-1 belongs to a class of nematode GSTs that is characterized by diminished peroxidase activity relative to other GSTs but elevated binding capacity for heme and related products. It is produced by adult hookworms and is thought to play a role in detoxifying heme and other breakdown products of the hookworm blood digestion pathway. Vaccination of laboratory dogs and hamsters with recombinant GST-1 results in reduced hookworm fecal egg counts and adult worm burden following challenge with infective larvae. Recombinant *Na*-GST-1 was expressed in *Pichia pastoris* and formulated with Alhydrogel according to current Good Manufacturing Practice. A dose-escalation Phase 1 clinical trial is being conducted in which 102 healthy Brazilian adults will receive either *Na*-GST-1 or the hepatitis B vaccine. Volunteers vaccinated with *Na*-GST-1 will receive 1 of 3 different dose concentrations (10, 30 or 100 µg) in 1 of 2 different formulations (*Na*-GST-1/Alhydrogel or *Na*-GST-1/Alhydrogel to which 2.5 µg of the Toll-like receptor-4 agonist, glucopyranosyl lipid A [GLA-AF], has been added as a point-of-injection preparation). Participants will receive 3 intramuscular injections at 2-month intervals. The study is being conducted in 2 parts, first in healthy adults with no history of hookworm infection or exposure and living in the urban center of Belo Horizonte, and then in individuals living in a hookworm-endemic area of the Brazilian state of Minas Gerais. All participants are being screened for IgE antibodies to *Na*-GST-1 and will be excluded if positive due to the previous experience with IgE-related urticarial reactions being observed upon vaccination with the recombinant *Na*-ASP-2 hookworm vaccine. Monitoring for vaccine-related solicited and unsolicited adverse events will be performed following each vaccination and until 12 months after the final vaccination. Antigen-specific IgG antibody responses will be measured in vaccinated participants at multiple time points throughout the study. Preliminary safety and immunogenicity results will be presented.

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INSIGHTS INTO THE SEROEPIDEMIOLOGY OF TOXOCARIASIS IN JAMAICA

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Jamaica has seen a decline in the transmission of soil transmitted helminths due to improved living standards. However, the status of toxocariasis is not known and its public health significance has not been quantified. The aim of this study was to determine the seroprevalence of toxocariasis and to establish the age-prevalence profile for the infection in Jamaica. One thousand serum samples submitted to the Microbiology laboratory at the University of the West Indies in Kingston, Jamaica for diagnosis of dengue were assayed for IgG antibodies to toxocariasis using the Toxocara-CELISA (CeLLabs, Sydney, Australia). The prevalence of anti-Toxocara IgG was 19.8 % and males (11.2%) were significantly more likely to be exposed than females (8.2%) [$\beta 2 = 3.67$; p=0.046]. Furthermore, there was no association between exposure to Toxocara and area of residence (rural vs. urban) [$\beta 2 =0.835$; p = 0.409]. Prevalence of infection peaked in young adults and declined thereafter. This pattern may be reflective of reduced exposure to infective stages as persons get older and mirrors the pattern seen for some soil transmitted helminths. Transmission of Toxocara appears to be active in Jamaica and further studies to elucidate its clinical and public health significance are indicated.

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PATIENT AND SITE LEVEL PREDICTORS OF LOSS TO FOLLOW-UP AND MORTALITY IN PEDIATRIC AND ADOLESCENT PATIENTS IN NIGERIA

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As ART access for children increases, understanding factors that contribute to suboptimal outcomes is important. This study describes pediatric and adolescent outcomes in Nigeria and explores the impact of site characteristics on loss to follow-up and mortality. A retrospective chart review was conducted from 2007-2011. Data collected included age, weight for age (z score), ART regimen, visit dates and mortality. Loss to follow-up was defined as not having a visit within 90 days of the last missed visit. A cross-sectional survey was administered at sites providing care. Information gathered included use of guidelines, staffing, service integration, laboratory and pharmacy capacity. We performed descriptive analyses and examined associations with survival and loss to follow-up using Cox proportional hazards and logistic regression. 1,431 children were randomly sampled from 23 sites. Mean age at initiation was 64 months (SD 51.8). Severe immunosuppression was noted in 45% (644) at enrollment. Mean z score was -1.1 (SD 4.3). Median duration of follow-up was 24.8 months (IQR 29.8). Mean time from eligibility to ART initiation was 26.2 weeks (SD 44.4). Mortality during the period of follow-up was 4.4% (95% CI: 3.4%, 5.4%). Mortality within 90 days of ART initiation was 2.0% (95%CI: 1.3%, 2.7%). Loss to follow-up was 19.1% (95%CI: 17.1%, 21.1%) and associated with site type [primary vs secondary or tertiary hospitals (p=0.009)] but not with any site characteristics or patient level factors. Mortality was associated with degree of immunosuppression at presentation (p=0.03), z score (p< 0.004), and reported lack of full implementation of the 2010 Nigerian Pediatric Guidelines (p=0.04). In multivariate analysis moderate (p=0.024) and severe immunosuppression (p=0.037) were found to independently predict mortality. Treatment outcomes in the Nigerian program are encouraging. Interventions to ensure earlier access to ART, decrease loss to follow-up, and promote full implementation of the most recent guidelines are warranted.

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EXPOSED BUT UNINFECTED: DOES HIV EXPOSURE ALTER IMMUNE RESPONSES IN INFANTS?

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The introduction of the Prevention of Mother to Child Transmission (PMTCT) programmes has greatly reduced vertical transmission of HIV. In the developed countries, mother to child transmission (MTCT) has decreased to less than 1%. PMTCT coverage is fast increasing in sub Saharan Africa and in many areas the use of 1 dose of nevirapine has helped reduce MTCT of HIV to less than 12%. This has in turn led to an increasing number of infants who are exposed but not infected (EUs). In addition, with the improved care for HIV infected individuals, more women are reaching the child bearing age, further increasing the numbers of EUs being born. Increasingly, there is evidence to suggest that EUs may represent an immuno-compromised group with an increased probability of infections. Vulnerability to infections is not only due to increased environmental exposure to pathogens, but may also be due to induction of tolerance following *in utero* exposure to: several drugs, an activated mother's immune system that distorts the cytokine mileu and soluble antigens that may modify the infant's developing immune system and the way that they respond to antigens. Current evidence largely tackles potential disruptions to the T cell compartment and associated immune responses. In this study we sought to determine whether B cell/ humoral responses in the EU infants are different from HIV unexposed (healthy) controls. To do this, HIV EU infants of less than 18 months and age-matched healthy controls were enrolled and prospectively followed up until 24 months of age. Comprehensive B cell phenotypic analyses was done and data categorized into different age brackets of the HIV EU and control infants ie., 3, 6, 9, 12,15 and 18 months. At 18 months of age, frequencies of vaccine antigen-specific (measles, tetanus and diptheria) memory B cells were determined by ELISPOT assays. Antibody levels, and the associated isotype distributions and avidity were determined against the same vaccine antigens. Preliminary data will be discussed.

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ASSESSING ADHERENCE TO COTRIMOXAZOLE PROPHYLAXIS USING SELF-REPORT AND PILL COUNT AMONG HIV EXPOSED CHILDREN IN A SEMI-URBAN DISTRICT IN MALAWI

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Improved Prevention of Mother to Child Transmission (PMTCT) interventions have shown to reduce the risk of HIV infections among children. In HIV exposed children, prevention of HIV opportunistic infections is especially important given their susceptibility to severe illness and death. Cotrimoxazole prophylaxis given to these children until they stop breastfeeding and exclusion of HIV, has been an important intervention in preventing bacterial and other adverse health outcomes. HIV exposed children must adhere to cotrimoxazole prophylaxis to achieve optimal responses. Understanding adherence to cotrimoxazole prophylaxis in a population perceived to be 'healthy' is important in designing strategies for compliance. This study determines the rates and factors associated with adherence among HIV exposed children receiving cotrimoxazole prophylaxis. We conducted cross-sectional interviews with guardians of HIV exposed children participating in a large cohort study

of 500 HIV exposed and 500 non HIV exposed infants in a rural district in Malawi.Two approaches to assessing adherence were used: Self report and pill count done by the research team. A total of 315 interviews took place. Seventy percent (n=222) of the children as reported by guardians were adherent to cotrimoxazole prophylaxis for the past month before interviews. However, when pill count was used, only 59% (n=185) indeed adhered (p<0.001). Some of the reasons for non-adherence ranged from forgetting (25%), running out of the medication (30%) and being away from home (20%). For those who reported running out of medications as the reason for non-adherence, 20% was a result of sharing the drugs between the child and the parent. Factors associated with adherence included having a working mother [OR=3.04 (95% CI: 1.22, 7.56)] and having a mother with higher educational level [OR=0.22 (95% CI: 0.09,0.53)]. Self reported adherence to cotrimoxazole is high among HIV exposed children. However, objective measurements are necessary to ensure full compliance to prophylaxis if these children are to achieve optimal benefits from cotrimoxazole.

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STATUS OF HIV SEROLOGY AMONG PULMONARY TUBERCULOSIS PATIENTS: THE PATTERN OF PULMONARY TUBERCULOSIS AND THE CHARACTERISTICS OF PATIENTS - MARCH 2011

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Tuberculosis (TB) is a leading cause of death among people with HIV. In 2009, there was an estimate of 380,000 deaths due to TB among HIV patients. 78% of TB/HIV co-infection cases reside in sub-Saharan Africa, HIV prevalence is as high as 80% in some countries. Therefore, the prevalence of HIV infection in the study population of TB was determined. Clinical, laboratory and radiological presentation of TB were analyzed and compared between HIV sero-ve and HIV sero+ve patients. Observational case-finding hospital based study was done on 60 TB patients, performed in 3 hospitals in Sudan. Interview based questionnaires and medical records were used for data collection. Prevalence of HIV infected TB patients among the study population was 16.7%. The study revealed that 50% of the HIV+ve TB patients were younger than 30 years. There was no major sex difference between HIV+ve and -ve TB patients (P=0.905). However, males were predominant among the whole study population of TB patients. 60% of HIV+ve patients originated from the North, where as the origins of the HIV-ve patients were more or less equally distributed (P=0.012). Clinical presentations of HIV+ve and -ve TB patients were similar and the differences weren't statistically significant. When comparing the lab findings; +ve sputum smear was found more common among HIV+ve patients (70%vs.54%) and a +ve PPD test was also more common among HIV+ve patients (75%vs.50%). A clear CXR was the only statistically significant difference between the 2 groups, being more common in HIV+ve (40%vs.6%) (P=0.002). The study concluded that the prevalence found was low in comparison to most of the countries in the region but high in comparison to developed countries. The main differences between HIV+ve and -ve was a +ve sputum smear, +ve PPD test, and a clear CXR, all of which were more common in HIV+ve TB patients. Therefore, the TB/HIV programme should be strengthened; starting by knowing the exact incidence of TB/HIV among the population, implementing better diagnostic tools and more researches to be conducted on a larger scale.

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HIV/AIDS INFECTION AND HIGH-RISK BEHAVIORS IN A PARAGUAYAN MILITARY POPULATION

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We conducted a research study between July 2005 and January 2006 on 1,248 Paraguayan active duty military volunteers in order to evaluate HIV seroprevalence and sexual behavior risk factors. Participants provided a blood sample for HIV testing and answered an anonymous survey about sexual behavior and risk factors. This information, as well as demographic data (age, rank, gender), was evaluated using a multivariate analysis. The median age of first sexual intercourse was 16 years, with no statistically significant difference between military ranks (H, p>0.05). Only 14.8% (CI 95%: 12.9 to 16.9) of participants reported condom use with every sexual encounter. Military students used condoms the most. Participants older than 45 years, compared with younger participants, had a fourfold (AOR 4.29) increased risk of not using condoms. Males were less likely to use a condom, more likely to practice anal intercourse, and had more sexual partners than females. Officers and non-commissioned officers (NCOs) were found to have a twofold (as measured by adjusted odds ratio, AOR=1.96 and 2.24 respectively) increased risk of having more than two sexual partners in the last month compared with students. Likewise, male personnel had almost a fivefold (AOR=4.75) increased chance of having more than two sexual partners over the last month compared with females. Both officers and NCOs were twice as likely as students to practice anal intercourse. By asking different questions about sexuality, we observed that some participants who did not identify themselves as homosexual actually participated in intercourse with individuals of the same gender, oftentimes considering themselves heterosexual because they took the insertive role in anal sex with another male. Despite the high-risk behaviors reported by those surveyed, we found only five cases of HIV among the entire population (0.4%; CI 95%= 0.15-0.89). Although HIV seroprevalence in Paraguayan active duty personnel was low, future educational efforts should focus on the high-risk behaviors and high-risk groups identified in this study.

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MICROBIAL COMMUNITIES, GENITAL HEALTH AND HIV-PREVENTION POLICY

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The most severe generalized HIV epidemics developed in complex health environments in which multiple morbidities interact to accelerate HIV spread. Substantial evidence indicates that malaria, urogenital schistosomiasis, untreated STIs, other reproductive-tract infections, and nutritional deficiencies increase HIV transmission and acquisition by increasing viral load, genital viral burden, or causing genital lesions or inflammation. HIV-prevention protocols, however, do not include interventions to reduce these cofactors that enhance sexual and vertical HIV transmission. HIV in multi-burdened populations is poorly understood. STI-HIV trials are confounded by high prevalence of other genital lesions caused by schistosomiasis, staphylococci, or streptococci. Lessons from plant pathology and microbial communities of the gut should inform research on disturbance of genital mucosa and epithelia to understand sexual transmission of HIV. The protective ability of genital mucosa depends on microbial communities gravely disrupted by disease. Even with treatment for schistosomiasis or STIs, recovery of the genital environment in which sex occurs exhibits hysteresis - delayed restoration of integrity of

protective mucosa. As with STI trials, RCTs of schistosomiasis treatment for HIV prevention will face confounding from multiple genital morbidities. Also, ethical obstacles (because there are safe, proven, cost-effective treatments available for STIs and genital schistosomiasis) require that controls be treated, thus making it unlikely that statistically significant effects on HIV incidence can be detected, in spite of the beneficial effects on treated individuals. Insistence on confirmation of treatment for cofactors (STIs, schistosomiasis) with an unachievable 'crucial experiment' for complex health problems, such as HIV in sub-Saharan Africa, is incorrect epidemiology and bad public health policy. Lack of full scientific certainty should not be used as a reason for postponing cost-effective measures to prevent threat of irreversible damage.

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EVALUATION OF A PRO-ACTIVE STRATEGY FOR MANAGING TB-HIV CO-INFECTION IN A UK TERTIARY CARE SETTING

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Management of TB-HIV co-infection is complicated by multiple interactions between diseases and therapies. We developed and evaluated a five-element strategy to: (i) treat co-infected patients in a single co-infection clinic, (ii) maximize use of first-line drugs, (iii) delay anti-retroviral therapy (ART) until 2 months post-TB treatment except in severe immunosuppression, (iv) commence efavirenz at 600mg daily with therapeutic drug monitoring (TDM), and (v) target treatment completion. Prospective cohort review over 5.5 years in a UK tertiary referral center. Fifty-six HIV-positive patients treated for TB were followed-up for a median 30 months. Main outcome measures were treatment completion, adverse events, IRIS, immunological and virological parameters and TDM for efavirenz. TB frequently presented with low CD4 counts (69% <200 cells/μL) and multiple, concomitant opportunistic infections. 15/56 cases (27%) occurred after ART commencement as 'unmasking IRIS'. First-line TB therapy and ART was used in 93 and 91% of cases respectively. Adverse events were common (55%), but caused no treatment interruptions. Treatment completion rates were 88% (49/56); four patients were lost to local follow-up and three (5.4%) died during treatment; no deaths were TB-related. Efavirenz TDM in patients receiving rifampicin showed very wide inter-individual variation (580 to 15,325 ng/L) but standard doses (600mg daily) achieved or exceeded therapeutic levels in 25/28 (89%). In conclusion, this study supports combined management for TB-HIV co-infected patients. Other opportunistic infections are common, but delaying ART to 2 months post-TB treatment did not seem to result in poor clinical outcomes. Although Efavirenz 600mg daily usually achieved satisfactory levels, TDM is recommended.

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HIV PATIENT WITH MUCOSAL LEISHMANIASIS TREATED WITH MILTEFOSINE IN COLOMBIA: A CASE REPORT

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Co-infection with *Leishmania* and Human Immunodeficiency Virus (HIV) has been reported in 34 countries around the world, mainly in southern Europe, where most visceral leishmaniasis cases. A few reports of cutaneous manifestations of leishmaniasis in patients infected with HIV have been reported. These groups of patients depict atypical manifestations of the disease and are at higher risk of leishmaniasis dissemination due to depletion of both humoral and cellular response of the organism. We report the case of a 27 year-old male patient, resident of Cáceres, Antioquia, in northeast Colombia, who was diagnosed with HIV with a history of cutaneous leishmaniasis years ago and then presented with Leishmaniasis in nasal mucosa. He was treated with miltefosine and after finishing treatment relapsed, needing to be treated with systemic pentavalent antimonials. This patient had a low CD4 count during the treatment and a bad compliance with the HAART therapy, which can be the cause of his relapse. There is a clear synergy between the two diseases, the unpredictable course and the challenge it poses to the treating physician. The atypical presentations of disease in *Leishmania* and HIV co-infection force to make a comprehensive approach to the diagnostic possibilities to reach the final pathology and to provide adequate and soon management. This is not only important to create more awareness within the scientific community, but to reinforce the fact that we need to count with a better therapeutic arsenal to treat tropical neglected diseases, since co-infection is increasing all around the world.

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KNOWLEDGE, ATTITUDE AND PRACTICE OF MALARIA PREVENTIVE MEASURES AMONG HIV POSITIVE AND HIV NEGATIVE PREGNANT WOMEN IN SOUTHWESTERN NIGERIA

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The pregnant woman is more prone to malaria than her non-pregnant counterpart with adverse pregnancy outcomes. HIV infection further increases the susceptibility of the pregnant woman to malaria and its consequences. Between July 2009 and August 2010, 1742 pregnant women [HIV +ve -497 (28.5%); HIV -ve 1245 (71.5%)] were enrolled at first antenatal clinic attendance in a tertiary hospital in southwest Nigeria. A self/investigator-administered questionnaire was used to collect information. Haematocrit and presence of malaria parasitemia were evaluated. Chi-squared was used to investigate association between categorical variables and ANOVA for continuous variables. Level of significance was set at β= <0.05. The mean age (±sd) was similar among the two groups of pregnant women. Prevalence of malaria parasitemia [12.7% versus 6.5%; β=<0.0001] and geometric mean parasite density [24,940 versus 8,550; β= 0.144] was higher among HIV +ve women. Mean haematocrit [30.8%±4.5 versus 34.25%±4.02; β=<0.0001] was significantly lower among HIV +ve pregnant women compared to HIV -ve women. Significantly more HIV +ve than HIV -ve women did not think that malaria was preventable [β= <0.0001]. Less than 12% of the study population [HIV -ve women more than HIV +ve women [10.6% versus 1.1%; β= <0. 0001] knew that malaria parasite was the causative agent for malaria while a larger proportion knew that mosquitoes were the vectors that transmit malaria [HIV -ve versus > HIV +ve; β= <0. 0001]. HIV -ve women were also significantly better informed about anti-vector measures and malaria chemoprevention [IPT, proguanil]. Although significantly more HIV -ve women had heard about ITN and were willing to use ITN (β= <0. 0001]. ITN ownership was similar in both groups of pregnant women (44.2% -HIV +ve; 40.2% -HIV -ve; β= <0.154). 24.9% and 20.1% (β= <0.098) of HIV +ve and HIV -ve women claimed to have slept under an ITN the night before the survey. In conclusion, prevalence of malaria parasitemia is significantly higher among HIV +ve than HIV -ve women. Inadequate knowledge and use of malaria preventive measures among pregnant women and especially HIV +ve pregnant women recorded in this study is a matter of concern. There is a need for a robust information, education and communication programme on malaria for all pregnant women in southwest Nigeria.

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PREVALENCE OF HIV INFECTION IN RELATION TO DEMOGRAPHIC/OBSTETRICS DATA AND MALARIA INFECTION AMONG PREGNANT WOMEN IN CENTRAL NIGERIA

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HIV infection has been found to increase the incidence and severity of clinical malaria. In non pregnant adults, it has been found to roughly double the risk of malaria parasitaemia and clinical malaria. A total of 200 pregnant women attending ante natal clinic in Jos University Teaching Hospital were sampled after obtaining informed consent. In relation to age, HIV infection was more prevalent among pregnant women aged between 12 and 19 years (64%, 95% C.I:50.7 - 77.0%) followed by women aged between 20 and 29 years (62.2%, 95% C.I: 52.3-72.1%). HIV was least common among the ≥ 40 year olds (40.0%, 95% C.I: 30 - 83.0%). There was however, no statistical difference (P>0.05) in HIV infection among the different age groups (Cal β = 0.8134 <Tab β 0.05 df_3 = 7.815). On parity, HIV was more prevalent among the primiparous pregnant women (72.6%, 95% C.I: 62.4 - 82.8%) followed by those who have had two children (59.3%, 95% C.I: 46.2-72.4%). The least prevalence was recorded among those who have six or more children (42.9%, 95% C.I: 6.2-79.6%). There was also no significant difference among the different parity levels (Cal β = 3.7388 < Tab β 0.05 df_3 = 7.815). In relation to gestational level, HIV infection occurred more among those in their 3rd trimester (66.7%, 95% C.I: 48.9 - 84.5%) followed by those in their 2nsd trimester (63.2%, 95% C.I: 54.6 - 71.8%). The least HIV prevalence was among those in their 1st trimester (50.8%, 95% C.I: 38.0-63.6%). There was no statistical difference (P>0.05) between the various gestational levels (Cal β = 1.2132 < Tab $\beta_{0.05}$ df_2 = 5.991). On coinfection of HIV and malaria, 26/41 (63.4%, 95% C.I: 48.7-78.1) of those infected with malaria also had HIV infection, while 94/159 non malaria patients had HIV infection (59.1%, 95% C.I: 51.5-66.7%). There was however, no statistical difference (P>0.05) in HIV prevalence between those infected with malarial and those who were not (cal β = 0.0617<Tab β 0.05 df_1 = 3.841).The public health significance of these findings are discussed.

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ANTIRETROVIRALS VS. STANDARD ANTIMALARIALS/ CO-TRIMOXAZOLE AS MALARIA CONTROL DRUGS IN HIV PATIENTS

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Previous studies found an increase in malaria among HIV patients in some regions. Also, malaria has been implicated as an enhancer of HIV replication and infectivity. One hundred HIV positive adults were recruited in Lagos, Nigeria. Drug history was ascertained by oral interview and review of clinic records. Blood samples were collected and laboratory tests carried out to obtain blood groups, Genotype, Malaria parasitaemia, CD4 counts, and Viral load. Malaria parasitaemia was significantly less among those on ARVs (Lamivudine, Zidovudine, Nevirapine) compared with those not on ARVs; 9.65 vs 13.3%(p=0.006). Patients placed on single course Artemisinin Combination Therapy, Sulphadoxine/pyrimethamine and Chloroquine within the last 28 days were without parasitaemia. Though 60% of patients on Co-trimoxazole had malaria parasitaemia this drug appeared to cause increase in CD4 values as average malaria parasite density declined. Our findings suggest that the criteria which govern use of these ARVs and antimalarials in malaria endemic regions need to be reviewed, promoting an earlier commencement of ARVs.

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TARGETING NEGLECTED DISEASES BASED ON RATIONAL APPROACH DESIGN; PROOF OF CONCEPT: NOVEL PEPTIDES FOR INHIBITING LEISHMANIASIS

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For over 20 years, we have developed short peptide inhibitors of protein-protein interactions between signaling enzymes, such as protein kinase C (PKC), and its scaffold protein, receptor for activated C-kinase (RACK). These short bioactive peptides are highly selective and effective in several animal models of human diseases. Some of these peptides were tested in humans and were shown to be safe. RACK interacts with and regulates multiple signaling enzymes that have key cellular functions. The RACK ortholog in *Leishmania*, called LACK, is not well characterized but is functionally critical. Parasites in which LACK was knocked out are not viable, and parasites that express low levels of LACK fail to parasitize even immune-compromised mice. Because of its homology to RACK, we assumed that LACK also interacts with multiple signaling enzymes in the parasite and might be a key scaffolding protein involved in essential signaling processes. Furthermore, LACK is found in both amastigotes and promastigotes of *Leishmania*. Therefore, we predicted that LACK is a good drug target, and we developed novel peptides aimed at inhibiting LACK interactions with LACK-binding proteins. Peptides were developed based on a sequence homology search and structural studies and were conjugated to TAT-derived peptide for drug delivery. When used to treat L. amazonensis promastigote cultures for 24 hours, some peptides resulted in growth inhibition with IC_{50} of approximately 10 μM. Furthermore, these peptides inhibited infection of macrophages by L. amazonensis promastigotes. The peptides are non-toxic to macrophages. Therefore, without any knowledge on partner proteins of LACK, we were able to design presumed inhibitors of LACK's function and affect the parasite's viability. Our method is likely applicable to design other anti-parasitic drugs.

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POLYMERASE CHAIN REACTION DETECTION OF *TRYPANOSOMA CRUZI* IN SUGAR GLIDER (*PETAURUS BREVICEPS*), HEDGEHOG (*ATELERIX ALBIVENTRIS*) AND CHIMPANZEE (*PAN TROGLODYTES*) USING ARCHIVED TISSUES

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We report a PCR based method to detect *Trypanosoma cruzi* organisms in formalin fixed paraffin embedded blocks from 3 sugar gliders, 2 hedgehogs and 1 chimpanzee which were previously diagnosed as *T. cruzi* positive by histopathology. Heart samples from animals referred to the Zoo/Exotic Pathology Service (West Sacramento, CA) were fixed in formalin, and embedded in paraffin blocks using conventional methods. For the PCR assays samples were obtained from the paraffin wax blocks using a 3-mm tissue punch (Acuderm. Inc, Fort Lauderdale, FL) and de-paraffinized overnight in xylene. DNA was extracted using the DNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's specifications. PCR was carried out using primers TCZ1 and TCZ2 and platinum *Taq*

polymerase (Invitrogen) under touchdown conditions. The PCR products were stained with ethidium bromide, visualized under UV illumination and photo-documented. Although *T. cruzi* has previously been reported in chimpanzees and cynomolgus monkeys, this is the first time this organism has been detected in sugar gliders and hedgehogs. In one hedgehog no *T. cruzi* organisms were found by histopathology but cardiac tissues were positive by the PCR method, demonstrating the higher sensitivity of the PCR method. Successful use of DNA from formalin-fixed, paraffin-embedded blocks is important because most pathology laboratories routinely archive tissue blocks. These archived paraffin embedded tissues can be used for further studies on the prevalence of this disease..

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INNOVATIVE SERUM-FREE MEDIUM FOR *IN VITRO* CULTIVATION OF PROMASTIGOTE FORMS OF *LEISHMANIA* SPECIES

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We described a comparatively simple medium formula (CML) using common, available and reasonably priced ingredients that could be used in place of medium that requires calf serum enhancement for cultivation of *Leishmania* promastigote forms. This medium equivalently supported the growth of parasites at rates comparable with those obtained with serum supplemented RPMI-1640 medium. *Leishmania* promastigotes reproduced in CML exhibited moderate to high infectivity capacities when tested against J774 macrophage cell line. No significant difference was noted between *Leishmania* strains cultivated in the newly modified medium and those grown in RPMI-1640 medium in their cells infectivity and replication potentials. The use of new CML can easily take the place of other biphasic or liquid media because of its easy preparation and instantaneous use, reasonable price, availability of ingredients, and its long shelf life, which is 30-45 days. The fact that this medium is similar to other culture media as far as durability and quantity of produced parasites might give it an advantage over the other currently used media.

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IDENTIFICATION OF INHIBITORS OF *TRYPANOSOMA CRUZI* INTRACELLULAR AMASTIGOTE REPLICATION BY FLUORESCENCE-BASED *IN VITRO* IMAGING ASSAYS

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Chagas disease (CD), caused by the protozoan parasite *Trypanosoma cruzi* causes over 15 million deaths per year and Chagas Heart Disease is the leading cause of infectious myocarditis in the world. CD, endemic to South and Central America, is designated a neglected tropical disease by the World Health Organisation and the National Institute of Health. Treatment of CD is limited to the nitroheterocyclic drugs benznidazole and nifurtimox that cause severe side effects and show very poor efficacy against the second, chronic phase of the disease. The search for new, more effective and less toxic drugs is a continuing development in CD research. To discover new active compounds against the parasite we have developed an imaging- based assay to enumerate *T.cruzi* intracellular amastigotes following exposure of *T.cruzi* infected 3T3 host cells to compounds or known control drugs. The assay technology utilises nuclear and cytoplasmic markers to fluorescently stain both the parasite and host cells, detected with a High Content imaging system and analysed with a developed script. With the use of 3T3 fibroblasts as the host cell, of which growth is not effected by *T. cruzi* early stage infection (as reported previously), host cell survival can also be estimated as a primary determination of compound mediated cytotoxicity in the one assay. This assay was utilised to determine the activity of a compound library of FDA approved and biologically active compounds. The most successful chemical candidates for further biological evaluation will be outlined. The development of a downstream assay relevant to human infection to assess compound activity against *T.cruzi* amastigotes internalised in primary human heart fibroblasts, along with a trypomastigote imaging-based assay will be presented. Although trypomastigotes are not the most clinically relevant form in consideration of the disease state, activity against this motile infective form would be favourable. These assays will be utilised to further profile the activity of selected compounds.

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USE OF 16S RRNA GENE UNIVERSAL PRIMER PAIR AS A PCR AMPLIFICATION POSITIVE CONTROL DURING THE DIAGNOSIS OF *TRYPANOSOMA CRUZI* IN ARCHIVED TISSUES FROM 4 MAMMALIAN SPECIES

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Trypanosoma cruzi infects a variety of mammalian species including humans, pets, and wild animals. To develop a PCR-based method for the detection of *T. cruzi* we tested archived tissues from chimpanzees, cynomolgus monkeys, hedgehogs and sugar gliders and characterized 16S rRNA as a DNA positive control that would make it possible to distinguish between PCR failure and truly negative results. The traditionally used PCR primer sets like β-globin and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) could not be used due to the species wide genetic differences. The16S rRNA primer set successfully amplified DNA from primates, marsupials and hedgehogs suggesting it is a potential universal DNA positive control primer for PCR-based test for *T. cruzi*. The priming sites of this primer set are sufficiently conserved among mammalian species and this primer set has been used before for species identification among vertebrates.The sequence of the 16S rRNA is: L2513 Forward 5'-GCCTGTTTACCAAAAACATCAC-3' and H2714 Reverse 5'-CTCCATAGGGTCTTCTCGTCTT-3'

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IDENTIFICATION OF NOVEL ANTI-LEISHMANIAL CHEMOTYPES THROUGH A SYSTEMATIC SCREENING OF KINASE INHIBITOR LIBRARY

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Leishmaniasis is caused by different species of protozoan parasites and is transmitted by the bite of an infected sandfly. Leishmaniasis is endemic in 88 countries with 350 million people at risk. Current therapies are limited by high cost, toxicity, compliance, parenteral administration, and cold storage. We have identified lead chemotypes with activity against leishmania through a systematic screening of 34 diverse structural classes from a large library of kinase inhibitors. The compounds were screened for *in vitro* activity against *Leishmania major, L. donovani* (promastigotes and axenic amastigotes) and for cytotoxicity in a mouse fibroblast cell line (L929). After four rounds of screening, with analog selection based on activity and cytotoxicity, two structurally distinct compound series were identified with IC_{50} values ranging from 30 nM to >5 μM. The top compounds were further evaluated in an intracellular assay. Mouse macrophage cells, infected with either *L. major* or *L. donovani* strains with an integrated luciferase reporter, were treated with compounds for 72 h and IC_{50} for parasite inhibition was determined by luminescence. Metabolic stability in the presence of mouse microsomes and cellular permeability in an MDR1-MDCK cell line was also assessed. Based on activity against intracellular parasites, metabolic stability and permeability assessments, four compounds were evaluated in a mouse model of *L. donovani* at 2 independent sites. Efficacy was calculated based on

reduction in liver infection as compared to untreated infected animals. Reduction in liver parasites of up to 43% was observed. Based on these results, we have initiated a SAR program to synthesize new analogs, targeting maintained or improved potency, but with improved ADME properties.

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REAL-TIME *LEISHMANIA* GENUS MASTER MIX: A STABILITY AND PLATFORM COMPARABILITY STUDY

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Leishmaniasis is a disease cause by the bite of a leishmaina infected sand fly of the species, *Phlebotomus* or *Lutzomyia*. Leishmaniasis is endemic in 88 countries and 10-15 million individuals are infected worldwide. Sand flies are native to the tropical and subtropical regions[1] where many American and allied soldiers are stationed. Therefore, it is necessary to have a molecular detection method for conducting *Leishmania* surveillance in the field. The Walter Reed Army Institute of Research has produced a "bulk" lot of *Leishmania* Genus real-time PCR master mix using a wet chemistry that has been validated in accordance with College of American Pathologists standards for use on the SmartCycler® instrument (Cepheid; Sunnyvale, CA). This bulk master mix was divided into 450µL aliquots and stored at -20°C. A verification study using a Limit of Detection (LoD) was performed to compare the bulk master mix to a newly prepared lot of master mix, and these results served as the T_0 time point for the stability study. Overall, results from the stability study indicate that the bulk *Leishmania* Genus master mix is stable when stored at -20°C. However, the Joint Biological Agent Identification and Detection System (JBAIDS, ITI; Salt Lake City, UT) is the Program of Record PCR platform utilized by the armed forces. Consequently, a LoD study was performed using the bulk lot of master mix to determine whether this wet chemistry would be compatible with the JBAIDS instrument. The following serial dilutions (400pg/µL, 40pg/µL, 4pg/µL, and 0.4pg/µL of *L. tropica* EP 139) produced positive results with average crossing point (Cp) values, which were three Cp values apart. A sensitivity and a limited specificity study were performed using both the SmartCycler and JBAIDS platforms, in which both platforms produced comparable results. Based on these results, the bulk lot of *Leishmania* Genus master mix is compatible with the JBAIDS. We foresee this assay to be used by armed forces in the field, which will have a significant effect on vector surveillance.

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CLONING, EXPRESSION AND PURIFICATION OF *LEISHMANIA DONOVANI* ANTIGEN FOR THE DIAGNOSIS OF VISCERAL LEISHMANIASIS

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Microscopic observation of amastigotes in splenic or bone marrow biopsies and serology including rK39 dipstick test are tools commonly used for diagnosis of visceral leishmaniasis (VL). However, bone marrow and splenic aspiration are painful and risky procedures and serology with rK39 dipstick can yield false positive responses in 20-32% of endemic healthy individuals. Identification of additional *Leishmania donovani* antigens could increase the specificity of noninvasive serologic testing for active VL. Therefore, we screened promastigotes soluble proteins using western blotting with a series of serum specimens from patients with acute VL. Western blots revealed a protein of molecular weight 70kDa (BHUP1), recognized by sera from VL patients but not healthy controls. Mass spectrometry of the gel-purified protein revealed the antigen as *L. donovani* HSP70. The full length *hsp70* gene of 1959 nucleotides was determined, cloned and expressed as a His-tagged fusion protein, purified, and retested. Antibody against this protein were detected in more than 96% of serum samples from patient with VL but not detected in sera from the endemic and non endemic control persons. Cross-reactive responses of sera from subjects with different disease like malaria and tuberculosis revealed the BHUP1 antigen test is highly sensitive for VL, but specificity was too low to differentiate from other infectious diseases

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INVESTIGATING TRYPANOTHIONE AS A BIOMARKER FOR TRYPANOSOMAL INFECTION

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Trypanosomatids are protozoan parasites that cause three neglected human diseases: Chagas disease in Latin America, human African trypanosomaisis, and leishmaniasis in 38 countries. We are developing diagnostic tools based on the detection of the parasite-specific small molecule trypanothione (TSH_2). Trypanosomatids use TSH_2 to modulate their redox metabolism in conjunction with trypanothione reductase, which is orthogonal to the system of glutathione and glutathione reductase used in their mammalian hosts. We hypothesize that TSH_2 can be used as a biomarker in the development of diagnostics for the early detection of trypanosomatid diseases. A sensitive and, inexpensive point-of-care (POC) diagnostic for early detection would prove invaluable in the treatment and control of these devastating parasitic diseases. In our preliminary studies towards a POC approach, we have shown that the bisarsenical probe fluorescent arsenical helix (FlAsH) binder ethane dithiol (EDT) adduct can be used to detect the parasite metabolite TSH_2. Our work in buffer solution showed that FlAsH-EDT_2 has 0.4% of the fluorescence of the FlAsH-$(TSH_2)_2$ conjugate. We are able to detect the metabolite in extracts from rat serum. Our second generation of molecular probes outperforms FlAsH for this application. These probes have a lower limit of detection and faster rate of detection. Most encouraging was our observation that the metabolite is detectable in serum extracts using a simple handheld UV lamp. That detection indicates that arsenical probes could be valuable tools for the development of a low technology, inexpensive, and rapid POC diagnostic for trypanosomatid diseases. We are also exploring the detection of TSH_2 using more conventional antibody detection for seamless integration into existing clinical infrastructure. We will describe proof of concept experiments for the detection of TSH_2 from cultured *Leishmania* and *Trypanosoma cruzi* using these complementary approaches.

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EVALUATION OF THE ACTIVITY OF THE COMBINATION OF AZITHROMYCIN PLUS FLUCONAZOLE AGAINST *LEISHMANIA (V) BRAZILIENSIS* AND *LEISHMANIA (L) AMAZONENSIS* IN GOLDEN HAMSTERS

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The exploration of new oral therapeutic options for the treatment of American Cutaneous Leishmaniasis is a research priority with a potentially significant impact in patient management. We have demonstrated the activity of azithromycin PO against *Leishmania braziliensis* although inferior to meglumine antimoniate, whereas no activity on *L. amazonensis* was detected. In the current study, golden hamsters were infected on hind foot with $5x10^5$ metacyclic promastigotes of *L. braziliensis* or *L. amazonensis*. Five groups were established: azithromycin PO 400 mg/kg/day (A); fluconazole PO 80 mg/kg/day (F); azithromycin PO plus fluconazole PO

20 mg/kg/day (F/A); meglumine antimoniate IM 60 mg/kg/day (G) and untreated animals (C). The treatment was administered immediately after infection and for 28 days. Size of lesions was determined weekly. A week after finishing treatment, animals were sacrificed. Parasite load in skin was measured by limiting dilution assay and cultures of skin, lymph node and spleen were made. In the *L. braziliensis* model, there were no significant difference in the progression of size of lesions in G compared to F/A, except in week 5, one week after treatments were completed, when G performed significantly better. Lesion size was smaller in G vs. C from week 2 until the end of the study, and in G vs. A in weeks 4 and 5. Similarly, F/A showed smaller lesion size since week 3 comparing to C. There were no significant differences in paired comparisons of other groups. In the limiting dilution assay, G, F/A and A showed no differences between them, while they showed less parasite burden than F and C. There was no difference between F and C. Cultures of infected foot and lymph node were positive in almost all animals, while the positivity of spleen were 50% in C, 40% in F, 10% in F/A and 0% in G and A. In the *L. amazonensis* model, only G showed activity. In conclusion, fluconazole alone has no activity for *L. braziliensis* and the combination fluconazole/azithromycin appears to have no additional effect than azithromycin monotherapy.

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HIGHLY ACTIVE ANTI-*LEISHMANIA* H2 MOLECULE DESIGNED *IN SILICO* TO INHIBIT PTR1 AND DHFR

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There is an urgent need for finding better and more efficient treatments for leishmaniasis, which should be less toxic, oral administrated, price affordable, and mainly and most important: endowed with good efficacy. Sixteen molecules were synthesized after extensive *in silico* design and selection by their theoretical ability to inhibit PTR1 (Pteridine Reductase-1) and DHFR (Dihydrofolato-reductase). Then the molecules were tested in *in vitro* culture assay of *Leishmania mexicana* M379 strain. The therapeutic response of the newly synthesized compounds was studied in a mice model of heavy infection by *L. mexicana*. The selected compounds were orally administrated to infected mice. The therapeutic effect was evaluated following lession size, dermatosis, mice activity, mortality, longevity, and parasite presence in blood by using PCR with JW11 and JW12 primers. One molecule called H2 and its acetylated precursor, H2A, were selected due to their good ability to achieve a quick and effective *Leishmania* growth inhibition by death induction of the parasite since the first 30 min at a dose between 1-10 µg/mL. The compounds were found to have therapeutic effect in eliminating parasites from the skin and blood, stopping lesion size increase ($P < 0.05$, Student's t Test) reducing mice mortality and prolonging mice life ($P < 0.05$, Chi Square Tests) at doses of 0.1 and 1 mg/mL given *at libitum* in drinking water during 3 days and up to two weeks. No adverse effects were noticed at these doses. The complete elimination of parasites could not be reached yet, since PCR that became negative during treatment, detected parasites in blood two months after treatment end. In conclusion, compounds H2 and H2A are highly active in anti-*Leishmania* therapy. The complete eradication of the parasite has not been achieved yet, but there is still work on this matter to be done. Extending treatment length, making compound combinations or preparing soluble compound formulations could increase the erradication rate of the parasite.

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FUNCTIONAL AND PHENOTYPIC PROFILE OF CD4+ AND CD8+ T CELLS IN *TRYPANOSOMA CRUZI*-INFECTED CHILDREN SUBJECTED TO TREATMENT WITH BENZNIDAZOLE

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In this study, we sought to gain a clearer understanding of the relationship between parasite persistence and the maintenance of *Trypanosoma cruzi*-specific T cells by examining the functional profile of *T. cruzi* antigen-responsive T cells and T cell phenotypes prior and after treatment with benznidazole (BZ) of 25 *T. cruzi*-infected children in the indeterminate phase of Chagas disease. The evaluation of the functional status of parasite-specific T cells before treatment with BZ showed that the majority of the patients display *T. cruzi*-antigen responsive $CD4^+$ producing both IFN-γ and TNF-α T cells, indicating polyfunctionality of the parasite-specific T cell compartment. Increased frequencies of terminally differentiated effector ($CD45RA^+CCR7^-CD62L^-$) and effector memory ($CD45RA^-CCR7^-CD62L^-$) phenotype $CD4^+$ and $CD8^+$ T cells were also observed in *T. cruzi*-infected children compared with uninfected controls. Within 8 months of treatment with BZ, the levels of total early-differentiated memory ($CD45RA^-CD27^+CD28^+$) T cells increased while the levels of fully differentiated memory T cells decreased ($CD45RA^-CD27^-CD28^-$) in most patients. The expression of $CD127^+$ (IL-7 receptor) on $CD4^+$ and $CD8^+$ memory T cells was also increased following treatment. These changes in the phenotype of the overall T cell compartment were associated with a significant reduction in *T. cruzi*-specific antibodies, as assessed by conventional ELISA assays. Our results show a significant impact on the T cell compartment early after treatment with BZ which might be indicative of a reduction in parasite load after treatment of children in the indeterminate phase of Chagas disease. The high proportion of children displaying polyfunctional T cell responses specific for *T. cruzi* is compatible with a more competent immune status in these subjects compared with our former findings in adult *T. cruzi*-infected individuals, supporting our hypothesis that very long term chronic infection eventually results in T cell exhaustion.

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CLONING, EXPRESSION AND IMMUNOLOCALIZATION OF A *TRYPANOSOMA BRUCEI* CONSERVED HYPOTHETICAL PROTEIN

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The identification and cellular localization of novel proteins in *Trypanosoma brucei* will provide valuable information for the identification of new drug, vaccine or diagnostic targets. In this study, an ORF coding for a hypothetical protein, designated tmp10, was identified using bioinformatics tools predicting it to be a surface protein (Tmp10). Tmp10 was successfully cloned from *T. brucei brucei* GVR35, expressed and purified as a partial-length, His-tagged recombinant protein (rTmp10) in *E. coli* BL21 (DE3). When probed with polyclonal rabbit anti-rTmp10, a Western blot on T. brucei brucei whole cell lysate indicated that Tmp10 is a ~25kDa protein, whereas analysis by indirect immunofluorescence microscopy indicated that the protein might be localized on the surface of the parasite. Furthermore, when cultured *in vitro* with anti-rTmp10 serum, inhibition of parasite growth was observed and this inhibitory effect was dependent on both serum concentration and time of incubation. These results suggest that Tmp10 is a novel surface protein expressed endogenously by *T. brucei brucei*, and antibodies against it could provide

protective immunity against trypanosomiasis. The results also illustrate the merits of mining genome databases using bioinformatics tools as a cost-effective way to facilitate the identification of novel proteins.

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IMMUNE STATUS IN PATIENTS WITH CUTANEOUS LEISHMANIASIS WITH THERAPEUTIC FAILURE

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Currently little is described about the immunological aspects of patients with cutaneous leishmaniasis (CL) with resistance to the main therapeutic drug (Pentavalent antimonials). The study evaluated and compared the immune status the patients with therapeutic failure ("Resistant") and patients who responded successfully to treatment ("Sensitive"). Patients with CL living in Bolivia, an area highly endemic for *Leishmania* sp. were enrolled into the Resistant and Sensitive groups mentioned about. Measurement parameters of the immune response were: CD4+ and CD8+ T cells, production of IFN-γ and IL-13 as markers of Th1 and Th2 response respectively, by peripherical blood mononuclear cells (PBMCs) stimulated with Antigen soluble leishmania (SLA). PCR analysis was performed to typify sp. linages the leishmania parasites, isolated from skin lesions of patients Resistant: The result show that: CD4+ and CD8+ T cells were below normal values in both study groups, these values of CD4+ and CD8+ T cells in both groups showed no statistically significant differences. Resistant patients developed a strong IFN-γ response to SLA than Sensitive patients: Production of IL-13 remained low and similar in both groups. The characterization of strains isolated from patients Resistant identified to *L. brasiliensis* and *L. guayanensis*. These results show that: i) the specific immune response of resistant and sensitive patients is polarized toward TH1 ii) Values of CD4+, CD8+ T cells indicate an immunodeficiency in both study groups iii) Studies of Molecular Biology, showed predominance of *L. brasiliensis* in most clinical cases iv) The results do not fully explain the treatment failure in Resistant patients, hypothetically we are thinking about parasite-related factors (resistance genes).

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EVALUATION OF IMMUNOLOGICAL MARKERS IN PERUVIAN SUBJECTS ASSOCIATED WITH DIFFERENTIAL OUTCOMES OF *LEISHMANIA* INFECTION

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Many studies support the notion that cell-mediated immunity involving a Th1 cytokine pattern is important for a protective immune response against Leishmaniasis. In this study, we investigated the Leishmania-specific lymphocyte response and cytokine production in different clinical manifestations of American Tegumentary Leishmaniasis (ATL). Our aim was to assess immunological factors associated with the clinical outcome of ATL. PBMCs from 20 active cutaneous leishmaniasis patients (ACL), 17 cured cutaneous leishmaniasis patients (CCL), 8 asymptomatic individuals (ASY) and 14 endemic negative controls (ENC) were stimulated with total soluble Leishmania antigen (TSLA). Lymphocyte immunophenotyping for T-helper (TCD4), T-cytotoxic (TCD8), B (CD19) and NK (CD16/56) cell subsets were determined by flow cytometry in whole blood and stimulated PBMCs. In addition, Th1/Th2/Th17 cytokines were measured in culture supernatants. Asymptomatic individuals were defined as those whose IFN-γ production was above the cut-off value (Mean of 9 non-endemic negative controls + 2SD = 13.08 pg/ml). Only ASY individuals showed significantly higher T cell population after stimulation when compared with ENC ($p<0.05$). No statistically significant differences were found in TCD4 and TCD8 cells in stimulated PBMCs among the evaluated groups. Interestingly NK cells showed higher number in ACL and CCL in both whole blood and stimulated PBMCs, when compare with ENC ($p<0.05$). Statistically significant higher values were found for IFN-γ levels in ASY, CCL and ACL; for TNF-α and IL-10 in ACL; for IL-17 and IL-2 in ASY ($p<0.05$), when those levels were compared with ENC. For IL-2, the opposite was observed in ACL who showed lower production in relation to ENC ($p<0.05$). Given that TCD8 and TCD4 proportions were not statistically different among the evaluated infected groups, while IFN-γ levels were increased according to clinical status (ACL>CCL>ASY), we suggest that NK cells could be an important IFN-γ source. Besides IFN-γ, we suggest that high IL-2 production in ASY could be related to infection resolution

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EXPERIMENTAL INFECTION OF *SUS SCROFA* (DOMESTIC PIG) WITH *TRYPANOSOMA CRUZI*

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Trypanosoma cruzi is responsible for producing Chagas diseases in humans.Its pathophysiology is not yet well understood.Therefore, our goal was to assess the domestic pig as an experimental model to study Chagas disease resulting from infection with the Bolivia strain. Five 60day old mixed breed female pigs were randomly inoculated, four with the Bolivia strain and one with saline.Two animals were infected with $1x10^6$ trypomastigotes/kg b.w and $5x10^6$ trypomastigotes/kg.b.w administered intravenously, and two pigs received the same doses by the intradermal route.Microconcetration method was used to evaluate parasitaemia from 7 days post-inoculation(dpi). IgM and IgG were assessed by EAE-ELISA from serum samples. Necropsy was performed at 150 dpi and tissues were collected to determine tissue damage and presence of amastigotes by histology. Clot samples and tissues were analyzed by PCR (kinetoplast DNA of *T. cruzi*). All infected animals showed a parasite burden from 10 dpi.This decreased in three groups at 35 dpi; animals inoculated with $1x10^6$ trypomastigotes/kg body weight by the intravenous route showed a reduction in parasitaemia at 45 dpi.All infected animals were parasitaemia-negative from 50 dpi. IgM was detected from 5 dpi in 3/4, 4/4 were positive at 10 dpi. Peak in IgM occurred between 20 and 25dpi and was maintained until 55 dpi, being negative at 90 dpi. IgG increased from 20dpi until 75 dpi and remained elevated at the end of the experimental period. PCR detected positivity in blood from infected animals from 3 to 60 dpi; however, at 75 dpi only 2 animals were positive.One animal remained positive up to 150 dpi.Tissue PCR found 3/4 infected animals were positive cerebrally, 2 were spleen positive, and only 1 was positive in the small intestine. All were negative in the heart, kidney, and large intestine. Nests of amastigotes were not found in the tissues. Slight lymphocytic perivasculitis was observed in the pericardial and meninx area. Furthermore, the splenic capsule showed increased lymphoid nests and mild disintegration of the white and red pulp. A slight focal glomerulonephritis with perivasculitis,mainly lymphocytic, were observed in the renal tissue. The control animal was negative in all analyses performed. Our study concluded that the pig can be used as an experimental model for Chagas disease, and suggests that with increasing time post-infection, the pig can develop similar chronic pathologies to humans.

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MULTIPLEX REAL-TIME PCR FOR DETECTION OF SPECIES OF SUBGENUS VIANNIA AND *LEISHMANIA* (L.) *DONOVANI*

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Leishmaniasis can be caused by over 20 distinct species of the genus *Leishmania.* The diseases present a wide spectrum of symptoms which

may be confused with other etiological agents. Species identification is needed, especially in geographic regions endemic for both cutaneous and visceral forms of the disease. In this study we describe three real-time multiplex PCR assays for detection of several species of *Leishmania* of the subgenus *Viannia* and *L. donovani* complex. The 313 samples used in this study were divided in two groups. Group I: 220 DNA samples from blood and tissue from dogs and humans positive for several species of *Leishmania*. Group II: 93 DNA samples from blood, tissues and stools from humans that were negative for *Leishmania*, but positive for others parasites; i.e., *Plasmodium* spp., *Acanthamoeba spp., Balamuthia mandrillaris, Naegleria fowleri*, *Entamoeba histolytica, E. dispar, and Trypanosoma cruzi.* A generic probe targeting 18SrRNA labeled with the CY-5 was used to identify the *Leishmania* spp. at genus level. Specific probes targeting the *Leishmania* actin gene labeled with FAM and HEX were used to discriminate subgenus *L. Viannia* and *L.* (*L.*) *donovani* complex respectively. The reactions were performed on an ABI 7500 Real Time PCR System (Applied Biosystems). The CY-5 generic reaction was able to detect 59 out of 66 (89.4%) and 100 out of 110 (90.9%) isolates previously identified as positive for the subgenus *Viannia* and *Leishmania (L.) donovani*, respectively. Using the FAM reaction 60 out of 66 (90.9) of the subgenus *Viannia* isolates were detected and by the HEX reaction 104 out of 110 (94.5%) of isolates of *Leishmania* (*L.*) *donovani* complex were detected. No cross reaction among the specimens from subgenus Viannia and *L.* (*L.*) *donovani* complex were observed. Isolates of *L. (L.) amazonensis, L. (L.) mexicana* and *L. (L.) major* were positive only for the CY-5 probe. The other species belonging to the subgenus *Leishmania*, such as *L. (L.) tropica* and *L. (L.) aethiopica*, also tested positive for both marker CY-5 and HEX. The 93 samples negative for *Leishmania spp*. showed no Ct in all analyses performed. The multiplex PCR using the FAM and the HEX probes was effective in discriminating species of the subgenus *Viannia* and the *L* (*L.*) *donovani* complex. The results indicate that the method could be useful for simultaneous detection of *Leishmania* species from subgenus *Viannia* and *L. donovani* complex.

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EVALUATION OF STABILITY OF THE PHENOTYPIC AND GENOTYPIC CHARACTERISTICS OF WHO REFERENCE STRAINS AND CLINICAL ISOLATES OF *LEISHMANIA BRAZILIENSIS* AND *L. PANAMENSIS*

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Methodologies used in the study of *in vitro* drug susceptibility, diagnostic tests and identification of *Leishmania* species require the use of control reference strains that do not lose their phenotypic and genotypic characteristics after successive passages *in vitro*. The number of passages of the *in vitro* strains can identify *Leishmania* phenotypes with increased *in vitro* growth potential. This could affect the precision to establish the logarithmic growth phase and the phenotypic and genotypic characteristics of the parasite. The objective of this study was to evaluate the behavior of the growth curve, the isoenzyme and monoclonal antibodies profiles, the amplification and RFLP of the mini-exon and Hsp70 genes in WHO *L/ braziliensis* and *L. panamensis* reference strains as well as in 2 clinical isolates in different culture passages (1, 5 and 10). We found that behavior of growth in the logarithmic phase and the percentage of viable parasites in the reference species and in the clinical isolates was similar in passes 5 and 10. However the behavior in passage 1 was different, which can be explained because the parasite population is not in the same phase of growth at the beginning of the culture. The genotypic characterization through PCR-RFLP using molecular markers for the mini-exón gene and the HSP70 genes showed no differences between the subcultures studied. In conclusion, no differences were found in the phenotypic and genotypic characteristics evaluated in the reference strains and clinical isolates in passages 5 and 10 of culture. Therefore, given the phenotypic and genotypic stability, it is recommended to use cultures up to passage 10 *in vitro* studies.

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CALMODULIN INTERGENIC SPACER: A USEFUL MARKER FOR THE IDENTIFICATION AND CHARACTERIZATION OF *LEISHMANIA* SPECIES

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Calmodulin gene modulates the calcium metabolism in various cellular activities on tripanosomatides. Although this gen is evolutionary highly conserved in tripanosomatides, it has been demonstrated that the 3' UTR regions in *Trypanosoma cruzi,* present genetic variations capable to regulate the expression. Furthermore, mutations of calmodulin intergenic spacer have been demonstrated to be specific for *T. cruzi* major groups. In this study we demonstrate that the segment of calmodulin gene containing both Untranslated Regions (3' and 5'UTR), may be used as molecular marker to distinguish between *Leishmania* species, after a sequence analysis. *Leishmania* reference strains and clinical isolates from Panamanian patients were evaluated. A set of primers were designed based on the open reading frame of the calmodulin gene to amplify the 3' UTR, the intergenic region and the 5' UTR. As expected, *Leishmania Viannia* and *L. mexicana* reference strains appear to have similar calmodulin gene organization, which according to *L. braziliensis* genome sequence comprises three copies of the calmodulin orf and two intergenic spacer: 1.2 kb and 1.6 kb bases. Only two copies of this gen appear to be present in *L. chagasi* genome. PCR conditions were set up to favor the amplification of the smaller fragment of the spacer (~ 1230 bp). Sequence analysis of cloned PCR products revealed that this region presents genetic variations that clearly identified each of the *Leishmania* reference species evaluated. Parasites isolated from clinical samples were classified as *L. Viannia panamensi*s based on the mutations pattern observed. Our preliminary results indicate that the intergenic spacer of the calmodulin gene is a useful molecular marker for the identification and characterization of *Leishmania* species.

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A FAMILY OF SEVEN CYTOCHROME B5 REDUCTASES AS NOVEL THERAPEUTIC TARGET IN *LEISHMANIA MEXICANA*

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Leishmania parasites are opportunistic protozoan flagellates that cause devastating and often fatal diseases such as mucocutaneous or visceral leishmaniasis in much of the tropical and subtropical world. Emerging drug resistance is one of the problems in leishmaniasis treatment, contributed by enzymes involved in the detoxification of pharmacological agents and other xenobiotics. One such enzyme, cytochrome b5 reductase (Cb5r), has a high pharmacological significance owing to its role in fatty acid elongation, ergosterol (*Leishmania*) or cholesterol (human) biosynthesis, and cytochrome P450-mediated detoxification of xenobiotics. We have identified a new family of seven Cb5r isoforms in *L. mexicana*, in contrast to the one major isoform in humans and only two isoforms in fungi (CBR1, MCR1). Phylogenetic analysis revealed that one *L.mexicana* isoform, LmexCb5r-7, has closest homology to human Cb5r and fungal CBR1, while the other six isoforms form three separate independent clades. LmexCb5r-1 and 2 are most distant from human Cb5r and are located in tandem repeat on the same chromosome 22, while the other five isoforms are each located on separate chromosomes. We have cloned LmexCb5r-1 and 2 and expressed as recombinant His_6-tagged protein in *E.coli* for subsequent biochemical and pharmacological analysis. Furthermore we modeled the molecular structure of all seven LmexCb5r isoforms *in silico* based on the crystal structure of the mammalian Cb5r enzyme from human and rat for rational drug design. Biochemical analysis of recombinant LmexCb5r-1 and 2 revealed similar substrate affinities

for NADH (K_m = 21.3±1.9 μM and 23.5±5.1 μM, respectively) but 7-fold higher V_{max} value in LmexCb5r-1 compared to LmexCb5r-2. We are currently also characterizing the LmexCb5r-7 isoform closest to human Cb5r. Interestingly, human Cb5r has about 4-fold higher substrate affinity (K_m = 6μM) and approximately 500-fold higher V_{max} value compared to LmexCb5r-1, and it hence will be particularly interesting to characterize LmexCb5r-7 closest to the human enzyme.

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HIGH LEVELS OF FETAL ANEMIA AMONG NEWBORNS IN RURAL NORTHEASTERN GHANA ARE INDEPENDENTLY ASSOCIATED WITH MULTIGRAVID MOTHERS AND *PLASMODIUM FALCIPARUM* INFECTIONS IN CORD BLOOD

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Malaria during pregnancy entails a risk of severe anemia in the mother, and low birth weight in her baby, but the relationship between placental malaria and fetal anemia is unclear. We hypothesized that fetal anemia (FA, Hb <12.5 g/dL), seen in 22% of live births during 2007 in rural northeastern Ghana, was directly related to placental malaria, and could be reduced by Fansidar-based Intermittent Preventive Treatment during pregnancy (SP-IPTp). This was investigated retrospectively in a cohort of 2096 newborns using polymerase chain reaction to detect *P. falciparum* in their cord blood samples. Results were analyzed against characteristics of the mother and baby using multivariate logistic regression. We detected *P. falciparum* in 18% of cord bloods, mainly among first time mothers (OR = 1.57, 95% CI: 1.18, 2.08), low birth weight (LBW, <2500g) infants (OR = 2.16, 95% CI: 1.65, 2.84), and in babies born during wet season (OR = 3.071, 95% CI: 2.321, 3.921). Unexpectedly, FA was independently associated with deliveries by multigravidae (OR = 1.85, 95% CI: 1.30, 2.62), and associated with cord blood *P. falciparum* infections in their newborns (OR = 1.83, 95% CI: 1.27, 2.64). There was no significant effect of SP-IPTp seen on reduced levels of either LBW or FA, but the risk of cord blood malaria infection was significantly higher for infants of mothers who used no SP-IPTp (OR = 1.61, 95% CI: 1.13, 2.29), and drug effect was dose-dependent: Optimal SP-IPTp, based on three Fansidar doses, was significantly more protective than suboptimal IPTp (1-2 doses) against malaria infection (OR = 0.64, 95% CI: 0.49, 0.84). Fetal anemia in northeastern Ghana occurs at a disproportionately high rate in babies born to multigravid mothers and is exacerbated by malaria during late pregnancy when fetal oxygen demand is greatest. The importance of IPTp for pregnant multigravidae women, and its benefit for their babies has been questioned, and debated, but seems clear and well-justified in light of these results.

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PUTATIVE PARASITE-ENCODED ADHESIN EXPRESSED ON THE SURFACE OF *PLASMODIUM YOELII* 17X INFECTED RETICULOCYTES

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Adherence of *Plasmodium falciparum* infected erythrocytes to vascular endothelial cells contributes to pathology and disease severity. This adherence is primarily mediated by var gene-encoded *P. falciparum* erythrocyte membrane protein-1 (PfEMP-1), which is unique to *P. falciparum*. Red blood cells (RBCs) infected with other malarial parasites, including *Plasmodium vivax*, are also reported to adhere to vascular endothelium, although the parasite-encoded RBC surface proteins mediating this adherence are less well studied. Using the reticulocyte-restricted parasite *P. yoelii* 17X, we developed an adherence assay that allows for isolation of parasites from adherent and non-adherent reticulocytes. Using *P. yoelii* DNA microarrays, we identified six genes encoding putative adhesins whose expression was consistently up-regulated in parasites from adherent versus non-adherent reticulocytes. Members of the yir and pyst-a multigene families, which are predicted to encode RBC surface proteins in *P. yoelii*, were not included among these putative adhesins. We focused on PY04120, which encodes a ~193 kDa protein that is highly conserved across malarial species. We have expressed a 26 kDa fragment of the PY04120 protein in *E. coli* and have generated rabbit antisera. We can detect PY04120 protein in a *P. yoelii* -infected reticulocyte membrane protein (PyRMP) preparation and immunofluorescence using live, unfixed *P. yoelii* 17X infected RBCs (iRBCs) indicates that PY04120 is expressed on the surface of a subset of schizont-stage infected reticulocytes. Sera raised against PY04120 can also partially block adherence of P. yoelii 17X iRBCs to the mouse endothelial cell line bEnd.3. These results indicate that the interaction of PY04120 with endothelial receptors may contribute to adherence of iRBCs in host tissues. Studies to generate a PY04120 gene knock out in P. yoelii 17X to further characterize this putative adhesin are in progress.

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IMPACT OF HOST SERUM IRON ON THE ERYTHROCYTIC STAGE OF THE MALARIA PARASITE

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Iron is an essential nutrient for *Plasmodium falciparum*. The malaria parasite requires iron for DNA synthesis, glycolysis, pyrimidine synthesis, heme synthesis and electron transport. The host RBC contains 100fg (20mM) of iron, however, the majority of it is sequestered in heme which is incorporated into hemoglobin. Rather than releasing the iron from the host heme, the parasite synthesizes hemozoin, an inert crystal of heme molecules. There is no evidence, that the parasite has the heme oxygenase activity necessary to release iron from heme and there have been conflicting reports about whether or not the parasite is able to utilize human transferrin. No plasmodial iron storage proteins, siderophores or chelators have been identified. Thus, despite an obvious iron requirement, it remains unclear what iron source *P. falciparum* is able to acquire and utilize during the erythrocytic stage of infection. In the present study we report that the availability of extra-cellular iron in the form of Fe citrate and human transferrin impacts the growth of erythrocytic stage *P. falciparum*. We show that the bioavailable iron and reactive oxygen content of parasitized erythrocytes is dynamic and subject to the availability of extracellular iron. Additionally, using confocal microscopy, we study the binding of fluorescently labeled human transferrin to late stage *P. falciparum* trophozoites and schizonts.

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MRI OF THE BRAIN IN ADULT PATIENTS WITH CEREBRAL AND SEVERE MALARIA

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Magnetic Resonance Imaging (MRI) allows detailed study of the pathogenesis of cerebral malaria in living patients but its availability in malaria-endemic areas is limited. A high powered scanner allows

assessment of cerebral blood flow, blood volume, oedema, ischaemia, haemorrhages and blood brain barrier integrity; and by magnetic resonance spectroscopy (MRS) metabolite levels and neuronal injury. A recent study in African children using a 0.35 tesla scanner found gross abnormalities to be common, especially brain swelling and ischaemia. There is accumulating evidence that the pathogenesis of cerebral malaria differs between adults and children. In adults, there have been several case series of MRI in patients with cerebral malaria but no systematic study. We performed a descriptive study of the morphological and functional changes of the brain using high-powered MRI and MRS in patients with severe and cerebral malaria. Patients were enrolled from 2009-2011, in Chittagong Medical College Hospital, Chittagong, Bangladesh. Sequences included T1, T2, Flair, DWI, GRE and magnetic resonance spectroscopy (MRS) including brain lactate levels. Scanning was done as early as possible within maximum 48 hours of admission. All patients also had a full clinical assessment including blood tests for haematology, biochemistry, acid-base status, lactate parasitaemia and HRP2. Retinal photography was performed to assess malaria retinopathy. 43 adult patients with severe malaria (30 with cerebral and 13 with noncerebral severe malaria) were enrolled, 26 were scanned in a 1.5 tesla and 17 in a 0.3 tesla scanner. 12/43 (28%) were fatal and 20/43 (47%) had hyperlactaemia. 80% of patients had abnormalities on 1.5 tesla MRI. These were mostly mild and included subtle cerebral oedema, ischaemia, mildly raised brain lactate and choline levels and venous congestion. Abnormalities were commoner and more marked in patients with cerebral malaria but less prominent than those found in Malawian children. The cerebral oedema found was thought not severe enough to cause coma. Ischaemia on DWI was mostly restricted to those with coma. This suggests cerebral oedema is not an important contributor to coma in adult cerebral malaria and the pathogenesis may be different from in children. Obstruction of the cerebral microcirculation by sequestered parasites is likely to be the cause of the brain ischaemia.

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BIOLOGICAL DISORDERS IN SUB SAHARAN CHILDREN (SENEGAL) WITH UNCOMPLICATED *PLASMODIUM FALCIPARUM* MALARIA TREATED BY ACTS

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Biological disorders are frequent in children acute uncomplicated *Plasmodium falciparum* malaria but not well studied. Several abnormalities can be present before the infection. Others as started at the beginning of the disease. These disorders can be worsened by drugs used for the treatment (drug toxicity). The objective of this study was to analyze biological parameters (hematologic and biochemistric) for children with acute uncomplicated *P. falciparum* malaria at the enrollments and after antimalarial treatment. Data on age, hematologic parameters (hemoglobin levels or hematocrit, platelet counts), biochemistry parameters (Alanine amino transferase, Aspartate amino transferase, bilirubine and creatinin) and treatment were collected and analysed. Univariate and multivariate analysis were done at day 0 and day 7 after treatment in overall patients and taking in account ACT used for treatment (ASAQ, AL, ASMQ, DHA-PQ). Data for 720 patients under 10 years were analyzed. Mean age of the patients was 9.4 years. Anemia was found in 72.78% (524/720) at D0 versus 80.14% (577/720) at D7 (p=0.001). Thrombocytopenia was present in 386 patients (53.61%) at D0 versus 47 patients (6.53%) at D7 (p=0.03). Regarding ALAT and ASAT, it appears that levels were higher at D0 compared to D7 (p=0.001). Same results were obtained in comparing bilirubine (72.78% at D0 vs 397 55.14% at D7) and creatinine (p=0.001). Positive impact of ACT's was not found in improving anemia between D0 and D7: 68.18% versus 79.26% for AL (n=352 p=0.0001); 68.3% versus 60% for DHA-PQ (n= 120, p=0.0001); 78.12% versus 88.12% for ASMQ (n=160, p=0.001) and 87.50% versus 96.59% for ASMQ (n= 88, p=0.004). Decrease and a return to normal of liver enzymes and creatinine were more significant in DHA-PQ group following by ASMQ and AL. In conclusion, our results showed that hematological disorders are common at the beginning of acute uncomplicated *P. falciparum* malaria in children and they persist up to 7 days after treatment with different ACT's. However, these drugs didn't affect biochemistry parameters.

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TRANSCRIPTIONAL PROFILING VALIDATES MECHANISMS AND BIOMARKERS OF ERYTHROPOIETIC SUPPRESSION IN SEVERE MALARIAL ANEMIA

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Malaria is a major global cause of anemia. In malarial anaemia, dyserythropoiesis and erythropoietic suppression can prevent adequate compensation for erythrocyte destruction, but these processes are poorly understood. To study their mechanistic basis, we collected 286 bone marrow (BM) aspirates in the context of a large case-control study of anemia in Mozambique and examined a wide range of clinical, hematological, biochemical, immunological, microbiological and genetic markers. We developed novel flow-cytometric analysis and a transcriptional profile based on erythroblast-specific gene expression to quantifiy erythropoiesis. Both measures correlate well with classic morphological quantification in stained bone marrow smears (both $p<0.0001$). Whereas peripheral reticulocyte numbers showed no correlation with bone marrow erythropoiesis in this cohort, the red cell distribution width (RDW), a measure of variance in erythrocyte volume, was validated as a novel peripheral biomarker for this important response ($p<0.0001$). Using these measures of bone marrow erythropoietic differentiation, samples were categorized as having low or high erythropoiesis relative to hemoglobin levels. A first pass analysis revealed that both bacteremia and HIV infection were more frequent in samples with low erythropoiesis. Unexpectedly, infection with malaria parasites was equally common in samples with low and high erythropoiesis with high erythropoiesis linked to severe anaemia in the presence of hemolysis. These findings are currently being validated and extended using rigorous uni- and multivariate statistical testing. Erythropoietin (EPO) insufficiency has been debated as a cause of erythropoietic suppression. Our data showed a strong fit of EPO concentrations with Hb levels but not with the level of erythropoiesis ($r^2=0.65$ vs. 0.29). Thus, these data provide superior direct and peripheral biomarkers of erythropoiesis, differentiate several groups of erythopoietically suppressed bone marrow and provide a rich data set for the analysis of transcriptional footprints indicative of underlying mechanisms. Such findings may inform future therapies for this serious disease.

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COMMON RED BLOOD CELL POLYMORPHISMS AND *IN VITRO* GROWTH OF *PLASMODIUM FALCIPARUM*

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It has been almost 60 years since it was proposed that the high frequencies of hemoglobinopathies seen in Africa and Asia were the result of selective pressure in response to *Plasmodium* species (The malarial hypothesis). Abundant evidence exists to support hemoglobin S (HbS) and to a lesser extent for hemoglobin C (HbC) and hemoglobin E (HbE) as examples of hemoglobinopathies conferring a reduced risk of severe malaria depending on an individuals' heterozygous state. The exact mechanism for this protection remains unclear. Studies have suggested

that the invading parasites cannot survive within variant red blood cells due to a number of different possible mechanisms. However, variations in in-vitro cultivation procedures make comparisons between studies problematic. We believe that oxidative stress may play a role in parasite survival in hemoglobin variant red blood cells. We describe and compare the *in vitro* growth of *P. falciparum* in red blood cells from individuals with defined hemoglobin (Hb) β chain variants, HbA, HbC, HbD/G, HbE, HbF, HbS, in a controlled oxygen atmosphere. Flow cytometry was used to enumerate infected cells after 72 hours of culture. Under low oxygen concentration (2%), we found a marked decrease in parasite growth in both the HbAS and the HbF RBC cultures as compared with HbAA. While growth in HbAC RBCs was slightly inhibited and no consistent pattern in parasite growth was seen in cultures using the HbD/G variant. Our results using HbAE red blood cells were inconclusive. Future experiments will be done under various oxygen concentrations. Our studies will help to better understand the complex interaction between malaria parasites and human red blood cells.

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SINGLE CELL GENOMICS FOR MALARIA PARASITES

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Malaria parasite infections are composed of millions of individual parasite cells. Infections frequently comprise multiple genotypes or species, and many parasite species are not possible to culture in the laboratory. As a consequence, next generation sequence data from malaria infections often comprises a soup of different sequences and accurate inference of component parasite haplotypes is problematic. To dissect the genetic composition of these complex mixtures, methods capable of genotyping (and phenotyping) single parasites would be ideal. Using approaches adapted from the cancer and bacterial literature, we have developed single cell methods – isolating single cells using fluorescence activated cell sorting (FACs), followed by whole genome amplification and array based genotyping or Illumina sequencing - to generate genomic data from single infected red blood cells. To demonstrate the utility of these methods we made mixtures of *Plasmodium falciparum* 3D7 and HB3 laboratory parasites lines, and then isolated and genotyped single cells from these mixtures. We are now further refining these methods to examine the genetic composition of natural infections containing *P. falciparum* and *P. vivax*.

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DEVELOPMENT OF A *PLASMODIUM CHABAUDI* RODENT MODEL SYSTEM FOR PLACENTAL MALARIA

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Pregnancy associated malaria (PAM) is associated with placenta pathology and poor pregnancy outcome but its mechanism is poorly understood. PAM has been studied in rodents for a long time now, but we still do not have a good existing rodent or non-human primate model. Here we propose a rodent model system for *Plasmodium chabaudi* infection during pregnancy in C57BL/6 mice and in African thicket rats; the latter are the natural rodent hosts for malaria. In the process we have also tried to study the pathogenesis and immune responses during placental malaria in these animals. We looked at the pathogenesis of the malaria infection before and during the pregnancy using two different strains of *P. chabaudi*, the AS and the CB. The mice when infected with the AS strain did not show any parasite recrudescence during the pregnancy but the mice when infected with the CB strain of *P. chabaudi* seems to develop chronic infection and did show parasite recrudescence during the pregnancy. We also got some interesting results when we transferred our understanding from these mice into the thicket rats. The development of these models and our findings from them will open new insights into the understanding of malaria during pregnancy.

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DEPLETION OF CD8 T CELLS DELAYS CLEARANCE OF PARASITEMIA AND INCREASES ANEMIA IN A RAT MODEL OF MALARIAL ANEMIA

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MSP7 is a malaria parasite gene family used by blood stage merozoites to invade erythrocytes. It is conserved through the genus *Plasmodium*. We have previously shown that deletion of a single *msp7* gene, reduced anemia despite a small change (3 to 2%) in peak, peripheral parasitemia in rats infected with *Plasmodium berghei,* as reported previously. We now report that in the spleen, the peak parasite load is 4-fold higher for wild-type parasites relative to Δ*mps7* counterparts. This occurs at day 8 post infection. At d10, which is also the onset of anemia, the spleen parasite load was reduced by 40-fold for wild-type parasite infected rats, compared to a ten-fold drop for Δ*msp7* mutant-infected animals. Analysis of gene expression, suggested that at day 10, there is elevation of granzymes in the spleen of rats infected by wild-type parasites compared to Δ*msp7* mutants. Cellular analysis indicated no difference in NK cells but a two-fold elevation in number of CD8 cytotoxic T cells. Immunohistochemistry revealed that by day 10, the CD8 T cells accumulated principally in the red pulp zone. Depletion of CD8 T cells (but not CD4 T cells), had no effect on the rise of parasitemia, but delayed parasite clearance from the periphery, increased parasite load in the spleen, and exacerbated anemia. These data suggest that CD8 T cells control parasite clearance. Further, persistence of parasitemia increased anemia. Current studies are focused on the mechanism by which CD8 T cells eliminate infected erythrocytes and how MSP7 engages CD8 T cell dynamics in the spleen.

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SUSCEPTIBILITY OF OLD VS. YOUNG ERYTHROCYTES TO *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum is known to cause severe disease in humans and accounts for one million deaths per year. The virulence of this parasite has been in part attributed to its ability to promiscuously invade young and old erythrocytes. However, it has been observed that young red cells are more susceptible to invasion. The parasite is known to utilize the sialic acid on glycophorins as a main invasion pathway and a sialic acid-independent invasion pathway utilizing complement receptor 1(CR1) was recently identified. As erythrocytes age they lose glycophorins and CR1 which could account for their lower susceptibility to invasion. We hypothesized that if aged red cells are less susceptible to invasion by *P. falciparum*, this may be due to decreased availability of these receptors. To test our hypothesis erythrocytes were separated based on age using a discontinuous Percoll gradient. Differential susceptibility to invasion of each red cell population was tested *in vitro* using invasion assays with the

P. falciparum 7G8 strain. We observed no difference in the susceptibility to invasion among red cells of different ages and no shift in the use of receptors with age.

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SUSTAINING THE GAINS AND PROGRESSING TOWARDS MALARIA ELIMINATION IN KWAZULU-NATAL PROVINCE, SOUTH AFRICA

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Malaria transmission increased nearly 10-fold from 1995 to 2000 in KwaZulu-Natal Province, South Africa, due mainly to vector resistance to pyrethroids and parasite resistance to sulfadoxine-pyrimethamine. Transmission subsequently dropped by 77% between 2000 and 2001 and by 99% between 2000 and 2011 (from 41,786 cases to 598). This marked decline was due to a combination of interventions, including the reintroduction of DDT in 2000, introduction of ACTs in 2001, cross-border collaboration with Mozambique and Swaziland through the Lubombo Spatial Development Initiative (LSDI), and intensified surveillance. However, cases in KwaZulu-Natal have plateaued between 2007 and 2011 with an average of 509 cases reported each year. The lowest transmission was experienced in 2010 with 380 reported cases followed by an increase to 598 cases in 2011, of which 51% were classified as imported - 69% of which were from Mozambique, 20% were local, and 29% were unclassified. This upsurge is concerning and may be due in part to the cessation of the LSDI. To sustain the gains, collaboration with Mozambique must continue to reduce imported cases, coverage of interventions in the province must remain high, and insecticide and drug susceptibility must be monitored to avoid resistance. As part of South Africa's move toward elimination by 2018, KwaZulu-Natal must not only sustain efforts but also transform its malaria programme to eliminate malaria systematically from its transmission foci. With the lowest local transmission in South Africa (121 local cases in 2011), KwaZulu-Natal can be the first province to eliminate malaria through prompt and robust collection and use of information, focal IRS, proactive screening and treatment among high risk groups, and effective response to every malaria case in receptive areas. Maintaining a strong vertical programme especially for IRS to enable rapid and extensive intervention will be critical while working toward integration of surveillance, IEC, and case management into the broader health system to prevent reintroduction following elimination.

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DEVELOPING THE MALARIA ELIMINATION TOOLKIT: A MONITORING AND EVALUATION TOOL FOR CASE INVESTIGATION AND REACTIVE CASE DETECTION

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Active case detection (ACD) strategies are recommended by the World Health Organization and are implemented in many malaria endemic countries worldwide as a critical part of malaria elimination programs. Strategies include determining the origin of infection, case investigation, and responding to a locally acquired case of malaria, known as reactive case detection (RCD). RCD strategies differ across countries, ranging from screening and treating household members to whole communities of the identified index case, and may include forms of vector control. Due to the programmatic and financial considerations of implementing RCD, malaria control programs need to be able to measure the operational effectiveness and cost-efficiency of their RCD strategies, as well as compare their RCD strategies to those of other countries. We have thus developed a standardized tool to monitor and evaluate the technical and operational effectiveness and cost-efficiency of RCD in malaria endemic settings. With our evaluation tool, RCD effectiveness and efficiencies are measured with process and outcome indicators, such as time lapsed between case diagnosis and investigation; percentage of cases investigated; proportion of additional cases identified through RCD; and screening and treatment. The evaluation tool gathers qualitative survey data, which are collected from focus group discussions with surveillance personnel concerning information flow and implementation of strategies. Financial analysis of surveillance-related expenditures are analyzed to determine the primary cost drivers and operating costs for RCD, resulting in an estimate for total cost per investigation and the cost per additional case of malaria actively detected. Findings from the evaluation tool can inform and test new programmatic interventions within endemic countries to mitigate challenges and strengthen the RCD surveillance program. The evaluation tool will be piloted in low transmission countries in the Asia Pacific and southern Africa with the goal to expand its implementation on a larger scale in the future.

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ACTIVE CASE DETECTION TOWARDS MALARIA ELIMINATION: OPERATIONAL FINDINGS FROM MPUMALANGA PROVINCE, SOUTH AFRICA

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To achieve malaria elimination by 2018, South Africa's malaria control programme is prioritizing intensified surveillance efforts with strong emphasis on Active Case Detection (ACD). Mpumalanga is one of three endemic provinces in South Africa and has a functional ACD system. The National and Mpumalanga Malaria Programmes jointly evaluated the provincial ACD system to identify best practices and inform ACD efforts in South Africa. The evaluation was conducted in the highest burdened municipalities, Bushbuckridge and Nkomazi, and consisted of qualitative and quantitative data collection methods and analysis. Questionnaires were administered to all direct and peripheral ACD staff in early 2012, and quantitative data was extracted from the provincial Integrated Malaria Information System (IMIS) and measured against defined indicators. Case investigation teams, consisting of one case investigator and at least two assistants, are responsible for conducting ACD, including case investigation. From July to December 2011, each team in Bushbuckridge was responsible for a mean of 29 malaria cases, with a range from 21 to 41 cases per team, while each team in Nkomazi was responsible for a mean of 71 cases with a range of 22 to 145 cases per team. The median number of days from diagnosis to ACD in Bushbuckridge was 4 days and 6 days in Nkomazi. The median number of days from diagnosis to entry of the ACD forms into the IMIS was 14 days and 6 days in Bushbuckridge and Nkomazi, respectively. This latter discrepancy is most likely due to the data capture centre being based in Nkomazi. Best practices include ACD-dedicated supervisors and case investigation teams and the placement of assistants at high burden health facilities in Nkomazi to complete notification forms and facilitate ACD. To improve operational efficiency, the malaria programme should consider implementing mapping techniques, improving communication between Bushbuckridge and the data capture centre, and restructuring teams to optimize human resource capacity toward ensuring a robust ACD system for elimination.

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ASSESSING THE EFFECTIVENESS OF ARTEMISININ-BASED COMBINATION THERAPIES IN RURAL GHANA

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The WHO recommends Artemisinin-based Combination Therapies (ACTs) for the treatment of uncomplicated *falciparum* malaria. Post licensure studies to monitor effectiveness of new drugs when delivered in real life settings are often lacking in Africa. We assessed the effectiveness of ACT policy five years following its implementation in the middle belt of Ghana. Both quantitative and qualitative surveys were carried out to assess uptake of ACT within the Kintampo Health and Demographic Surveillance System between October 2009 and July 2011. Participants were residents who reported of fever/malaria with a two-week recall. Finger prick blood sample for malaria microscopy were also collected at randomly selected households and health facilities during patients exit interviews. The population prevalence of malaria parasitemia was 28.2% (95% CI: 25.7-30.4) and 37.1% (95% CI: 30.3-44.3) among children less than five years old. About 40.6% (95% CI: 38.1-44.0) of patients who reported fever sought health care within 24 hours. Only 46.9% (95% CI: 40.5-53.4) of fever cases were investigated for malaria. About 62.1% (95% CI: 55.6-68.4) of patients were over-treated with ACTs based on reference blood slide results. About 48.0% (95% CI: 42.9-53.2) of patients prescribed ACTs failed to comply with medication instructions. Negative perceptions associated with the use of ACTs were also reported by some communities. Limiting factors of the health systems and negative community perceptions are potential impediments to uptake and adherence to ACTs. These do not only erode the benefits of efficacious ACTs but has potential for development of resistant strain of malaria parasites.

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HIGH-THROUGHPUT ASSESSMENT OF THE INFECTIOUSNESS OF NATURALLY INFECTED GAMETOCYTE CARRIERS TO *ANOPHELES* MOSQUITOES

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The most important biological outcome measure in future vaccine or drug-based transmission reducing trials in endemic malaria areas is the proportion of infected mosquitoes in direct (membrane) feeding assays. These assays were previously found to give highly variable results and this is an issue that needs to be solved if it is going to be used in the evaluation of the efficacy of future GCP clinical trials. Here, we study the reliability of two different methods in determining the proportion of infected mosquitoes post membrane feed. Fifty gametocyte carriers of 2-12 years of age were asked to donate each a 5mL blood sample to be distributed over four minifeeders for experimental mosquito's infection. One hundred mosquitoes were allowed to feed on each feeder for exactly 15 minutes. On Day 7, fifty mosquitoes from each experiment were dissected for microscopic detection of oocyst in infected mosquitoes. The sensitivity of an ELISA based-detection method of circumsporozoite protein in oocysts was determined for mosquitoes on day 7, 10 or 14 after feeding. Overall and as detected by microscopic examination, 66% (33/50) of children infected at least one mosquito. In total, 14.12% (1,059/7,496) of the mosquitoes were infected with a mean of 7.76 (range 1-110) oocysts per midgut. ELISA-based detection of oocysts on day 10 and 14 was more sensitive than on day 7 post-feeding and was strongly correlated with microscopic examination of midguts. In conclusion, these findings suggest that early microscopic examination of midguts for determining the biological outcome measure in transmission reducing interventions may be less informative. ELISA-based detection of oocyst, a high-throughput method as compared to microscopy appears to be more sensitive and convenient for the evaluation of transmission control interventions in the context of malaria eradication.

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MODELING THE IMPACT OF FIRST- AND SECOND-GENERATION MALARIA VACCINE ROLL-OUT STRATEGIES

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Given the desire to develop new tools to pair with existing drugs and vector control for the purpose of eradicating malaria from the most endemic regions of the globe, the advent of new malaria vaccine candidates is encouraging. Even with present prospects of only partially protective immunity, pre-erythrocytic and gametocyte-blocking vaccines may be useful as components of multi-intervention eradication strategies in certain circumstances. We present a systematic study of the utility of potential malaria vaccines within the EMOD model, a comprehensive model of the vector life cycle coupled to a detailed mechanistic representation of intra-host parasite and immune dynamics. Over a range of vaccine efficacies and roll-out scenarios, the impact of introducing malaria vaccines, alone and in conjunction with large-scale ITN coverage, is demonstrated for a variety of transmission intensities and entomological behaviors and ecologies. We present reductions in incidence among the protected population as well as community-level reductions in transmission. Our results demonstrate the types of settings where first-generation malaria vaccines are likely to provide the greatest impact, and provide estimates of efficacy targets for second-generation vaccines as a function of desired impact.

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BALANCING INTERVENTIONS WITHIN FIXED-BUDGET MALARIA CONTROL PROGRAMS IN AFRICA

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Malaria control programs often work within a fixed budget. In order to maximize economic and social benefit, they must balance their efforts among a variety of tools (indoor residual spraying, insecticide-treated nets, and antimalarial drugs). We study how this balance changes across different levels of endemic transmission, vector behavior, intervention coverage, and regional income level. We use the stochastic agent-based EMOD model to quantify the impact of a program on malaria transmission, mortality and morbidity, and evaluate how costs may scale as a function of coverage for each type of intervention considered.

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STEPS TOWARDS MALARIA ELIMINATION: INTEGRATING POPULATION-WIDE TEST AND TREAT CAMPAIGNS INTO MALARIA CONTROL IN ZAMBIA

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The current Zambia National Malaria Strategic Plan (NMSP) 2011-2015 targets elimination of malaria in 5 areas/districts by 2015. Based on data from Malaria Indicator Surveys (MIS's) carried out in 2006,2008, and 2010, the NMCP stratified the country into 3 epidemiological zones. The National Malaria Control Program (NMCP) and partners used a DHIS 2 mobile phone system to increase information on malaria prevalence in specific district health facility catchment areas and the information generated from this system led to the identification of districts prioritized

for elimination. A step-wedge, health facility catchment randomized design was used to identify areas for inaugural campaigns conducted in two Southern Province Districts (Gwembe and Sinazongwe) in December 2011 and January 2012. Community Health workers (CHW) were trained on standard procedures for screening, testing with malaria rapid diagnostic tests (RDT:SD Bioline Malaria A Pf) and treatment (artemether-lumefantrine) and, working collectively through catchment areas, performed a screening census to identify RDT positives and treat infections. Across 10 health facility catchments, 52,374 people were listed, 47,516 (91%) were tested and 12,961 (27%) were malaria RDT positive. Catchment teams achieved on average 9.8 households tested per day and completed catchment screenings on an average within 30 days. Across the catchments, RDT parasite prevalence ranged from 1.2% to 39.9%. Households on average reported 0.65 ITNs (range: 0.26-0.99). A cost per infected-case tested was estimated to be $7.21 and the cost per treated case estimated to be $8.06. In conclusion, next-in-line strategies for reducing community malaria transmission should involve optimizing vector control and population-wide malaria infection treatments which in combination have the potential to push communities toward malaria elimination. Subsequent rounds of these campaigns will be evaluated to assess their effectiveness in reducing malaria burden.

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MOBILE RAPID REPORTING SYSTEM IMPROVING MALARIA MONITORING IN ZAMBIA

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Insecticide-treated nets (ITNs) are an effective tool in controlling malaria and a primary malaria prevention strategy for the Malawi National Malaria Control Program (NMCP). In October 2010, the NMCP conducted a mass distribution of ITNs on Likoma Island on Lake Malawi to rapidly scale up prevention coverage and this was followed by a repeat mass ITN distribution during 2011. Two censuses were conducted one year apart, in October 2010 and in November 2011, to assess ITN coverage and malaria parasite prevalence levels among local residents using similar questionnaires and test methods. Children under age five were tested to determine anemia (Hemocue® HB 105) and malaria parasite prevalence using both slide microscopy and rapid diagnostics tests (RDTs: Clearview® Malaria PF). 11,079 and 10,923 household members were listed on Likoma Island in the 2010 and 2011 censuses, respectively. The percentage of households with at least one ITN increased from 64% to 84% from 2010 to 2011. The percentage of children under age five reporting to have slept under an ITN the previous night was 45% in 2010 and 42% in 2011; pregnant women who reported sleeping under an ITN the previous night was 36% in 2010 and 34% in 2011. Malaria related disease burden decreased over the year, with severe anaemia (Hb<7 gm/dl) in children falling from 7.0% in 2010 to 4.2% in 2011; overall anemia declined from 59% to 54% and malaria parasite prevalence among children decreased only slightly from 9.2% in 2010 to 7.8% in2011. Prior to 2010, ITN distributions in Likoma Island were through routine health facilities targeting only children and pregnant women and had achieved only low coverage. Leading up to the development of the National Malaria Strategic Plan 2011-2015, the repeated population wide distribution of ITNs in 2010 and 2011 achieved and then further increased high household ITN ownership. Based on the two full population assessments of Likoma Island residents after the ITN distributions, reported ITN use the previous night and malaria parasitemia rates showed little change while severe anemia in young children showed the largest relative decline of malaria related health outcomes. The NMCP and its supporting partners have continued to use population-wide distribution of ITNs, have begun innovative education and advocacy at community level to ensure proper use of ITNs, and are exploring additional options to further reduce malaria transmission on the Island.

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FOLLOW-UP CENSUS BASED PARASITE PREVALENCE ASSESSMENT AND IMPACT OF INSECTICIDE-TREATED NETS (ITN) COVERAGE ON LIKOMA ISLAND, MALAWI

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Insecticide-treated nets (ITNs) are an effective tool in controlling malaria and a primary malaria prevention strategy for the Malawi National Malaria Control Program (NMCP). In October 2010, the NMCP conducted a mass distribution of ITNs on Likoma Island on Lake Malawi to rapidly scale up prevention coverage and this was followed by a repeat mass ITN distribution during 2011. Two censuses were conducted one year apart, in October 2010 and in November 2011, to assess ITN coverage and malaria parasite prevalence levels among local residents using similar questionnaires and test methods. Children under age five were tested to determine anemia (Hemocue® HB 105) and malaria parasite prevalence using both slide microscopy and rapid diagnostics tests (RDTs: Clearview® Malaria PF). 11,079 and 10,923 household members were listed on Likoma Island in the 2010 and 2011 censuses, respectively. The percentage of households with at least one ITN increased from 64% to 84% from 2010 to 2011. The percentage of children under age five reporting to have slept under an ITN the previous night was 45% in 2010 and 42% in 2011; pregnant women who reported sleeping under an ITN the previous night was 36% in 2010 and 34% in 2011. Malaria related disease burden decreased over the year, with severe anaemia (Hb<7 gm/dl) in children falling from 7.0% in 2010 to 4.2% in 2011; overall anemia declined from 59% to 54% and malaria parasite prevalence among children decreased only slightly from 9.2% in 2010 to 7.8% in2011. Prior to 2010, ITN distributions in Likoma Island were through routine health facilities targeting only children and pregnant women and had achieved only low coverage. Leading up to the development of the National Malaria Strategic Plan 2011-2015, the repeated population wide distribution of ITNs in 2010 and 2011 achieved and then further increased high household ITN ownership. Based on the two full population assessments of Likoma Island residents after the ITN distributions, reported ITN use the previous night and malaria parasitemia rates showed little change while severe anemia in young children showed the largest relative decline of malaria related health outcomes. The NMCP and its supporting partners have continued to use population-wide distribution of ITNs, have begun innovative education and advocacy at community level to ensure proper use of ITNs, and are exploring additional options to further reduce malaria transmission on the Island.

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VARIANTS WITHIN THE FCΓRIIIA-176A/C AND TOLL-LIKE RECEPTOR (TLR)-9 (-1237T/C) GENE PROMOTER IS ASSOCIATED WITH INCREASED SUSCEPTIBILITY TO PEDIATRIC SEVERE MALARIAL ANEMIA AND CIRCULATING IFN-Γ

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Plasmodium falciparum malaria is still one of the leading global causes of infectious disease burden. In *P. falciparum* holoendemic transmission areas, such as in Siaya District in western Kenya, high rates of malaria-related pediatric morbidity and mortality is due to severe malarial anemia [SMA, hemoglobin (Hb)<6.0g/dL, any density parasitemia]. Since Toll-like receptors (TLRs) and Fc-γ receptors (FcγR) affect innate and adaptive immune responses, the functional association between polymorphic variants within FcγRIIIA (-176A/C, rs396991) and TLR-9 (-1237T/C, rs5743836) and susceptibility to SMA were investigated in children (n=301) presenting with acute malaria in a *P. falciparum* holoendemic transmission region in western Kenya. Hematological and parasitological

profiles were determined in all study participants. TaqMan 5′ allelic discrimination assay was used to determine FcγRIIIA-176A/C and TLR-9 -1237T/C genotypes. Circulating interferon (IFN)-γ levels were measured using Enzyme-Linked Immunosorbent Assay (ELISA). Multivariate logistic regression analyses controlling for potential confounders demonstrated that the presence of FcγRIIIA -176C/TLR-9 -1237T (-176C/-1237T) (OR; 2.05, 95% CI, 1.19-3.53; *P*=0.009) increased susceptibility to SMA two-fold while individuals -176C/-1237C were protected from SMA (OR; 0.36, 95% CI, 0.20-0.65; *P*=0.001). Consistently, stratification according to WHO definition of SMA (Hb<5.0g/dL, any density parasitemia) demonstrated that -176C/-1237T increased susceptibility (OR; 2.88, 95% CI, 1.61-5.17; *P*<0.0001) while -176C/-1237C protected from SMA (OR; 0.31, 95% CI, 0.14-0.68; *P*=0.004). Furthermore, the -176C/-1237C was associated with significantly higher circulating IFN-γ levels relative to non-176C/-1237C (*P*=0.014). Findings presented here demonstrate that variations in TLR-9 at -1237 and FcγRIIIA -176 are associated with increased susceptibility to SMA and functional changes in IFN-γ.

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VARIATION WITHIN THE INTERLEUKIN-13 (IL-13) GENE PROMOTER (-7402T/G AND -4729G/A) IS ASSOCIATED WITH SUSCEPTIBILITY TO PEDIATRIC SEVERE MALARIAL ANEMIA AND FUNCTIONAL CHANGES IN CIRCULATING IL-13

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Plasmodium falciparum malaria is still a major global cause of disease burden. In holoendemic *P. falciparum* transmission areas, such as western Kenya, severe malarial anemia [SMA, hemoglobin (Hb)<6.0g/dL, any density parasitemia] results in high rates of pediatric morbidity and mortality. Since interleukin-13 (IL-13) has been associated with pathogenesis of different infectious diseases, including *P. falciparum* malaria, the functional roles of polymorphic variants within IL-13 gene in conditioning susceptibility to SMA and reticulocyte production index (RPI; an effective measure of erythropoietic response in anemic children), were investigated. The relationship between the IL-13 variants -7402T/G (rs7719175) and -4729G/A (rs3091307) and susceptibility to SMA (Hb<6.0 g/dL, any density parasitemia) and RPI (<2) was investigated in children (n=387) with *falciparum* malaria from a *P. falciparum* holoendemic transmission region in western Kenya. Hematological and parasitological profiles were determined in all study participants. The IL-13 -7402T/G and -4729G/A genotypes were determined using TaqMan 5′ allele discrimination assay. Circulating IL-13 levels were measured using Enzyme-Linked Immunosorbent Assay. Multivariate logistic regression analyses controlling for potential confounders demonstrated that -7402G/-4729G (GG) (OR; 1.55, 95% CI, 1.01-2.37; *P*=0.044) were associated with increased susceptibility to SMA while -7402T/-4729A (TA) was associated with favorable erythropoietic response (RPI) (OR; 0.53, 95% CI, 0.29-0.98; *P*=0.043). In addition, carriers of the TA haplotype had significantly higher circulating IL-13 levels relative to non-TA haplotype (*P*=0.005). Findings presented here demonstrate that variation in IL-13 promoter at -7402T/G and -4729G/A is associated with increased susceptibility to SMA and functional changes in circulating IL-13 levels.

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A NEW MEMBER OF THE *PLASMODIUM VIVAX* TRYPTOPHAN RICH ANTIGEN (PVTRAG) MULTIGENE FAMILY SHOWS HIGH IMMUNOGENICITY AND RED BLOOD CELLS BINDING ACTIVITY

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Genes coding for the tryptophan-rich proteins are a part of multigene families identified in various *Plasmodium* species. Our recent studies have focused on the characterization of such antigens of the *P. vivax* parasite where we have investigated the possible role of these proteins in the elicitation of immunological responses and erythrocyte binding activity. Here, we report the immunological responses of a bacterially expressed recombinant and refolded His-fusion 32 kDa *P. vivax* tryptophan-rich antigen (PvTRAg32) amongst the *P. vivax* clinical isolates, its role in the erythrocyte binding activity, and its localization in the parasite. PvTRAg32 contains unusually high percentage of tryptophan residues (10.7%), which are positionally conserved with its orthologues in P. yoelii (PypAg1 and PypAg2) and *P. falciparum* (PfTryThrA and PfMATRA). Thirty four of the 40 (85.0%) *P. vivax* isolates showed seropositivity to recombinant PvTRAg32 by ELISA. The Mean ± SD values of OD for P.*vivax* subjects and naïve individuals were 1.02 ± 0.36 and 0.26 ± 0.11, respectively. In the Western blot analysis, majority of the subjects studied (n=44) showed high reactivity to the purified PvTRAg32. Immunoelectron microscopy and Immunofluorescence assays reveal PvTRAg32 is localized in micronemes and expressed at various erythrocytic stages of the parasite. Sequence analysis of the clinical isolates from various parts of the country shows that pvtrag32 is highly conserved and erythrocyte binding assays show significant binding as compared to the control antigens. High immunogenicity, conserved nature, and red cells binding activity could implicate this antigen as a multi subunit vaccine candidate against *P. vivax*.

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FILTER PAPER COLLECTION OF *PLASMODIUM FALCIPARUM* MRNA FOR DETECTING SUBMICROSCOPIC GAMETOCYTE DENSITIES

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Accurate sampling of submicroscopic gametocytes is essential for epidemiological studies to identify the infectious reservoir and enable appropriate application of disease control methods. Using gametocyte mRNA as a target enables sensitive detection, but requires careful handling of samples due to the labile nature of RNA. Filter papers can be used for collecting RNA samples but their capacity to withstand adverse storage conditions has not previously been rigorously tested, and evaluated with molecular detection methods QT-NASBA and RT-PCR in parallel. Three gametocyte dilutions; 10 g/µl, 1.0 g/µl and 0.1g/µl were spotted onto filter papers that were stored under; frozen, cold chain or tropical conditions for up to 3 months. RNA was extracted and detected by QT-NASBA and RT-PCR. Detection was higher from the Whatman 903 Protein Saver Card compared to the Whatman FTA Classic Card, by both techniques (p<0.0001). Storing papers at temperatures warmer than-80°C lead to a significant decrease in detection for the Whatman 903 Protein Saver Card, when evaluated by QT-NASBA p=0.025 and RT-PCR p=0.088.This study indicates that RNA can be recovered from filter papers, that the Whatman 903 Protein Saver Card is superior for this purpose to the Whatman FTA Classic Card for RNA sampling, but that storage above >-80°C will result in a loss in detection. Our findings indicate that in absence of optimal storage conditions, filter papers provide a useful alternative to low density gametocyte sampling.

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GENE EXPRESSION CHANGES AND SEQUENCE VARIATION IN *PLASMODIUM FALCIPARUM* CHLOROQUINE ADAPTED STRAINS

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The current scope of chemotherapy in the treatment of malaria has been limited due to the development of drug resistance to a number of

highly effective drugs including the use of chloroquine. Mutations in the *Plasmodium falciparum* gene loci Pfmdr1 N86Y and Pfcrt K76T are known to confer drug resistance to chloroquine. The role played by genetic mutations versus changes in gene expression levels and gene copy number has been highlighted, but the overall effect of each mechanism, singly or in interaction, to cause variable changes that mediate drug sensitivity still remains to be clearly defined. To determine the of role altered gene expression levels and gene copy number of the target loci Pfmdr1 and Pfcrt in mediating drug resistance in P. f. chloroquine adapted strains that is not associated with single nucleotide polymorphism gene mutations. P. f. strains adapted via cultures to different concentrations of chloroquine will be monitored for decreased drug sensitivity via the 3H-Hypoxanthine incorporation method at different drug exposure levels. SNPs primarily at Pfmdr1 86 and Pfcrt 76 will be determined via a novel Real Time-PCR allelic discrimination TaqMan genotyping assay and gene sequencing, respectively. Gene expression and copy number will be determined by a Real Time-PCR relative quantification assay. There is a significant change in the IC_{50} values of the *Plasmodium falciparum* strains beyond the drug exposure level equivalent to its IC_{30} an indicator of developing drug resistance. SNP analysis shows that the genotype of the strains primarily at Pfmdr1 86 (A, [N]) and Pfcrt 76 (A, [K]) remains unchanged over the course of the study in its wild type state. We next intend to look at changes in the level of gene expression and copy number, and compare its overall effect in the development of resistance to chloroquine versus SNP gene mutations. The course of drug resistance development in P. f. still remains highly uninvestigated as pertains to the specific manner and mechanisms by which it occurs. This may assist in understanding the molecular evolutionary mechanisms of anti-malarial drug resistance by linking genetic polymorphisms, gene expression levels and drug sensitivity and assisting in further monitoring and evaluation of malaria drug resistance in current treatment practices.

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THE HEME BIOSYNTHESIS PATHWAY IS NOT ESSENTIAL FOR *PLASMODIUM FALCIPARUM* DURING BLOOD-STAGE GROWTH

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Malaria parasites appear to have a functional heme biosynthesis pathway, although they potentially have access to the large amount of heme resulting from hemoglobin digestion in the digestive vacuole. They are believed not to salvage heme since almost all digestive heme is crystallized to form hemozoin. The generally accepted view in the field has been that the *de novo* heme synthesis pathway is essential for malaria parasites, and thus a good prospective drug target. Thus it came as a surprise that we were able to knock out genes for the first and the last enzymes, viz. 5-aminolevulinate synthetase (ALAS) and ferrochelatase (FC), of the malarial heme biosynthesis pathway. ALAS synthesizes 5-aminolevulinic acid from glycine and succinyl-CoA while FC inserts a ferrous iron into protoporphyrin IX to generate the final product, heme. Parasites of both knockout lines exhibited fully normal growth, suggesting the heme biosynthesis pathway is not essential or very important during the asexual blood stages. Since heme is the prosthetic group of several cytochromes (a-, b-, & c-types), which are components of the essential mitochondrial electron transport chain (mtETC), malaria parasites must apparently salvage heme, most likely from hemoglobin digestion. These results are remarkable because 1) they challenge the dogma that heme biosynthesis is essential in malaria parasites; 2) they support the idea that malaria parasites can also salvage heme; and 3) they may inform investigations involving antimalarial drug discovery. Further characterizations of these knockout parasites are underway to fully realize the implications of these unexpected findings.

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GENETIC MECHANISMS FOR AVIAN MALARIA HOST-SPECIFICITY

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A characteristic of emerging diseases is the switching of pathogens to novel hosts. In a time of global anthropogenic changes, it is important to study environmental influences on pathogen host-switching, and the genetic mechanisms responsible for host specificity in natural populations. While it is clear that habitat degradation can threaten bird populations, it is unclear how these alterations affect disease transmission and disease susceptibility. *Plasmodium*, the causative agent for malaria, has become an emerging disease in some organisms due to its ability to switch hosts. Avian malarias are unique because certain strains are host-specific; they can only infect one species of birds, but not another within the same family. While other strains are generalist; they can infect multiple species of birds. Furthermore, birds are inter- and transcontinental migrants and harbor well-characterized generalist and host-specific parasites. This makes them great models in research concerning parasite transmission and specificity. We aim to identify the genetic factors that contribute to and regulate the host-switching capabilities of pathogens, particularly in *Plasmodium* species that infect chicken and other birds. Specifically, we propose to characterize the erythrocyte binding-like family of genes extracellular binding domain region because they have been implicated in determining malaria host-specificity. We hypothesize that gene expression levels vary between generalist and specialist parasite, and that the genetic variations within the binding domain accounts for host-specificity and the spread of virulent, generalist malaria strains. Using high-throughput sequencing, we will profile the expression of mRNAs during *P. gallinaceum's* erythrocyte stage of infection. Our goal is to identify genes involved in host-specificity and determine the molecular differences that allow parasite transmission to a small or broad host range. This project will result in the first transcriptome, or analysis of total transcripts, of a malaria parasite infecting non-mammalian hosts. It will provide novel information on host-switching factors that shape pathogen virulence and threaten naive host populations.

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GENOME-WIDE VARIATION AND SELECTION SIGNATURES IN *PLASMODIUM FALCIPARUM* CLINICAL ISOLATES FROM A RURAL MALAWIAN POPULATION

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Implementation of large scale malaria interventions is bound to greatly disturb the natural environments of *Plasmodium falciparum*, by providing a selection landscape within which the parasite has to adapt. In this study, we have examined the resulting adaptive mechanisms to provide a map of genetic variation and signatures of selection from whole genome analysis of 89 uncultured *P. falciparum* clinical isolates from a Malawian population. We have catalogued SNPs, small insertions and deletions, and larger structural variants in our population. Our genome-wide analysis for selection signatures has revealed both known and novel vaccine and drug candidates. Understanding this variation against a background of intense malaria interventions will be key to controlling malaria and inform better use of the few interventions still available.

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ROLE OF POLYMORPHIC VARIATION IN *IL17* IN CONDITIONING THE CLINICAL OUTCOMES OF *PLASMODIUM FALCIPARUM* INFECTION IN AFRICAN CHILDREN

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In East Africa, children during the first two years of life are highly susceptible to severe malarial anemia [SMA, hemoglobin(Hb)<5.0g/dL]. We and others have found that genetic variation in innate immune response genes (IRGs) influences clinical outcomes in these children since their adaptive immune response has not fully developed. Among the IRGs, the role of interleukin (IL)-17, a pro-inflammatory cytokine responsible for neutrophil attraction to extracellular pathogens through anti-microbial peptide production, is largely unexplored in children with SMA. As such, we focused on several single nucleotide polymorphisms (SNP) in the *IL-17* promoter with a high minor allele frequency in the target population [-399A/G (rs3819024, A=0.68, G=0.32)and -1803T/C (rs677901, T=0.82, C=0.18)]. Multivariate logistic regression was performed in children (aged 3-36mos) infected with *Plasmodium falciparum* malaria (n=780) controlling for the confounding effects of age, gender, HIV-1 status, bacteremia, G6PD, α-thalassemia, and sickle-cell trait. Findings demonstrated that carriers of the -399G/-1803C haplotype had decreased susceptibility to SMA (OR=0.67, 95%CI=0.45-1.00, *P*=0.05). Additional analyses aimed at determining bone-marrow responses in the cohort revealed that the GT haplotype was associated with a lower reticulocyte production index (RPI <2, OR=2.22, 95%CI=1.01-4.87, *P*=0.05), while the AC haplotype was associated with a higher RPI (OR=0.54, CI 95%=0.36-0.79, *P*=0.002). Collectively, these results demonstrate that haplotypes in the *IL17* promoter condition susceptibility to SMA and reticulocyte production in this population.

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RELATIONSHIP BETWEEN HAPLOTYPES OF *IL13* PROMOTER POLYMORPHISMS AND MALARIA OUTCOME MEASURES IN KENYAN CHILDREN

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Plasmodium falciparum-associated severe malaria anemia (SMA) is a major cause of morbidity and mortality in sub-Saharan African children. The pathology of SMA is a consequence of the balance between pro- and anti-inflammatory mediators. Interleukin (IL)-13 is a powerful anti-inflammatory cytokine whose gene is located in a haplotypic block in the 5q31 cytokine gene cluster which also includes *IL3*, *IL4*, *IL5*, and *IL9*. We recently showed that the IL-13 pathway was associated with the development of SMA in Kenyan children. In addition, variations within the *IL13* promoter have been associated with several infectious diseases; including severe malaria among Thai adults. To further explore the role of IL-13 in malaria, we investigated the relationship between *IL13* -590A/G (rs2069743), -1257A/G (rs2069739), and -1456A/C (rs1881457) variants and malaria outcome measures (i.e., SMA and high-density parasitemia; HDP) among *falciparum* parasitemic children (n=850; aged 3-36 mos) presenting at the Siaya District Hospital, western Kenya. Children were dichotomized into either SMA (hemoglobin (Hb) <5.0g/dL; n=287] and non-SMA (Hb≥5.0g/dL; n=563), as well as HDP (HDP; ≥10,000 parasites/μL; n=573) and low-density parasitemia (LDP; <10,000 parasites/μL; n=277). Multivariate logistic regression model controlling for the confounding effects of age, gender, sickle-cell trait, HIV-1 and bacteremia status, showed no significant relationships between the haplotypes and SMA. However, the AGA haplotype was associated with a significantly increased risk of developing HDP (OR; 1.72, 95% CI 1.02-2.90, *P*=0.043), while carriage of the GAA haplotype was associated with significantly lower circulating IL-13 levels [median (IQR); 22.70 (34.56)] relative to individuals lacking the haplotype [28.45 (35.93); *P*=0.044]. Results presented here suggest that *IL13* variants may be important for conditioning susceptibility to parasitic burden, but not SMA, and underscore the complexity in discerning the role of the Th-2 cytokines in mediating malaria disease outcomes.

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EXTENDED HAPLOTYPES IN THE MIG (CXCL9) AND IP-10 (CXCL10) PROMOTERS INFLUENCE SUSCEPTIBILITY TO *PLASMODIUM FALCIPARUM*-ASSOCIATED SEVERE MALARIAL ANEMIA

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Plasmodium falciparum-induced severe malarial anemia [SMA, hemoglobin (Hb)<5.0g/dl] is a leading cause of morbidity and mortality in African children. However, the underlying genotypic traits that condition SMA have not been fully elucidated. Chemokines such as interferon gamma-induced protein-10 (IP-10/CXCL10) and monokine-induced by gamma (MIG/CXCL9) are typically increased during a type 1 inflammatory response. We recently observed that circulating levels of IP-10, MIG, and IFN-γ were significantly reduced in children with SMA compared to parasitized children with non-SMA (*P*=0.002, *P*=0.009, and *P*=0.019, respectively). To investigate the role of polymorphic variation in IP-10 and MIG on susceptibility to SMA, extended haplotypes were constructed using three promoter variants [IP-10 -1035G/A (rs4257674), IP-10 -1919G/T (rs4371639), and MIG -560G/A (rs6532083)] on chromosome 4. These particular variants were selected based on a high minor allelic distribution in the population. Multivariate logistic regression analysis (n=768; aged 3-36mos), controlling for covariates (age, gender, G6PD, HIV-1, bacteremia, α-thalassemia, and HbAS status) revealed that the ATA extended haplotype (-1035A/-1919T/-560A) was protective against SMA (OR:0.345, 95%CI: 0.225-0.530, *P*<0.001), and a favorable bone marrow response (RPI>2; OR:1.515, 95%CI: 1.045-2.197, *P*=0.028). In contrast, the extended GGG haplotype showed increased susceptibility to SMA (OR:4.087, 95%CI: 2.302-7.255, *P*<0.001) and decreased bone marrow responsiveness (OR:0.274, 95%CI: 0.138-0.543, *P*<0.001). Further analyses showed that carriage of the ATA haplotype was associated with increased circulating IP-10 levels (*P*=0.159), whereas carriage of the GGG haplotype was associated with reduced IP-10 (*P*=0.004) and MIG (*P*=0.156) levels. These results illustrate that variation in the IP-10/MIG extended haplotypes are associated with susceptibility to SMA and altered production of inflammatory mediators known to influence the clinical outcomes in children with malarial anemia.

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PEPTIDE NUCLEIC ACIDS (PNAS) AS A TOOL TO INVESTIGATE *PLASMODIUM FALCIPARUM* GENE FUNCTION

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Malaria remains a significant cause of morbidity and mortality worldwide. Drug resistance is widespread, and with a safe and effective vaccine still unavailable, new chemotherapeutic agents are required to ensure that cheap and effective treatment is widely available. To improve treatment options into the future we must improve our understanding of malaria parasite biology and identify new drug targets. A significant bottle-neck in the validation of targets for drug development has been the lack of robust methods to study gene/protein function, particularly when the gene of interest is essential to parasite growth and development. We have been investigating the use of Peptide Nucleic Acids (PNAs) as a tool to examine the function of proposed drug targets in *Plasmodium falciparum*. PNAs are DNA analogues that are assembled from a pseudo-peptide backbone. They bind to DNA and RNA via Watson-Crick base-pairing rules and as a consequence can change cellular gene expression. As PNAs lack a negatively-charged backbone their hybridisation to DNA/ RNA occurs without electrostatic repulsion and is stronger and faster than normal base pairing. Furthermore, PNAs are inert to natural proteases and nucleases and do not activate RNAase H. Our studies confirm that PNAs can be delivered into P. falciaprum asexual parasites and that these molecules can be used to investigate gene/protein function in *P. falciparum*.

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ESTIMATION OF WITHIN-HOST HAPLOTYPE FREQUENCIES OF *PLASMODIUM FALCIPARUM*

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The recent emergence of resistance to artemisinin derivatives reinforces the need for effective surveillance of antimalarial resistance. The spread of parasites that carry drug-resistant haplotypes from their point of origin is a major driver of antimalarial resistance. However, the accurate detection of the resistant haplotypes from patient samples is problematic: individuals can be infected with multiple parasite clones, especially in areas of high endemicity. When the multiplicity of infection (MOI) exceeds one, the allele sequences of the constituent clones at genotyped loci cannot be reconstructed. Consequently, statistical methods are required to deconvolute the allele sequences, infer distinct haplotypes and ascertain their frequencies from prevalence data. To address this problem, a Bayesian model for estimating haplotype frequencies has been developed. The model estimates haplotype frequencies based on prevalence data collected for one or more molecular markers known to been associated with antimalarial resistance. Prior knowledge of the MOI is not required. The model cycles over different, possible MOIs and, for each, calculates the likelihood of the data for potential estimates of the haplotype frequencies. The model quantifies the uncertainty in the frequency estimates using a Metropolis-Hasting Monte Carlo Markov chain algorithm. For each haplotype the model returns a distribution of frequency estimates from which the mean and its credible interval are derived and the average MOI for each sample. The model is then validated using simulated data sets for which the true haplotype estimates are known. We present results of the application of our algorithm and model to estimate haplotype frequencies for a set of historic data from Africa in which the prevalence of mutations associated with sulphadoxine-pyrimethamine resistance were obtained.

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MOLECULAR ANALYSIS OF *PLASMODIUM FALCIPARUM* INFECTIONS FROM CORD BLOOD SAMPLES OF NEWBORNS IN RURAL NORTHEASTERN GHANA

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Umbilical cord blood samples collected as part of a large (n = 2097) birth cohort study in rural northeastern Ghana were assayed by PCR to identify placental malaria infections and assess the impact of sulfadoxine/ pyrimethamine (SP)-based intermittent preventive treatment during pregnancy (IPTp). Cord blood *Plamodium falciparum* infections were 38% more prevalent in first time pregnancies and the IPTp target of 3 SP doses in the last six months of pregnancy was associated with 36% fewer cord blood infections in newborns. We hypothesized that: 1) malaria infections in births by primigravidae would reflect a greater clonal composition, owing to abundant available binding sites for *P. falciparum* in the placenta of first time pregnancies, and 2) *P. falciparum* infections in deliveries by mothers who did use SP would carry a higher frequency of point mutations associated with SP resistance. We analyzed five microsatellite markers from cord blood positive deliveries by primigravid and multigravid mothers that were matched for S/P doses and bednet (ITN) use. Tandem repeat polymorphisms in cord blood positives were determined by amplification of fluorescent-labeled PCR products which were then sequenced. Positive cord bloods were analyzed for single nucleotide polymorphisms (SNPs) in *P. falciparum* genes associated with drug resistance using a multiplexed microsphere-based suspension array platform. These analyses included dihydrofolate reductase (*Pfdhfr*) and dihydropteroate synthase (*Pfdhps*), key markers of sulfadoxine/ pyrimethamine (S/P) resistance, and the multidrug resistance protein 1 (*Pfmdr1*) and chloroquine resistance transporter (*Pfcrt*) genes. Mutation frequencies detected in *P. falciparum* from cord blood infections are being compared and analyzed against maternal doses of SP taken for IPTp and against a *P. falciparum* profile from this same location in 2001, before chloroquine was replaced by artesunate-amodiaquine, as the standard for uncomplicated malaria treatment and before SP became the standard for malaria prevention in pregnancy.

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A MORE ANCIENT ORIGIN FOR HIGH COPY NUMBER OF THE MEROZOITE SURFACE PROTEIN 3 (MSP3) FAMILY

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Gene families encoding antigens have been shown to be actors in many key processes of host cell invasion and immune evasion for the *Plasmodium* parasites that cause malaria. Lineage specific differences in gene copy number for these families are thought to be evidence of adaptation with consequences for pathogenesis, however these

differences are often elucidated from few or distantly related species. The ecological ubiquity of *Plasmodium* species, along with a growing richness of genomic data, allows more accurate insights about the evolution of gene families and adaptation. Previous comparisons hypothesized a *P. vivax* lineage specific expansion of the Merozoite Surface Protein 3 (MSP3) family. We show here that the macaque parasite P. cynomolgi, as well as other related species, vary in copy number and that some species exhibit copy number equivalent to that of *P. vivax*. This indicates that high copy number alone in the MSP3 array is not a result of adaptation to the human host by *P. vivax*, and predates its common ancestor with P. cynomolgi (estimated 2.36-5.27 mya). In addition, phylogenetic inference and synteny indicates that the MSP3 family as described for *P. falciparum* may be analogous rather than homologous to that of *P. vivax* and its related species. Variable patterns of genetic diversity, domain architecture and protein similarity ranging from 18 to 80% were observed among the putative *P. vivax* MSP3 members, suggestive of diversifying selection acting to drive the divergence of paralogs or reflecting gene duplication events at multiple time scales. High levels of silent and replacement polymorphisms between P. cynomolgi and *P. vivax* MSP3 genes point to rapid divergence of the family between even the closest related species. Our findings caution against the inference of adaptation based on copy number changes alone, and show the utility of placing lineage specific gene family changes in an evolutionary context, especially when the signal of homology is low.

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PFF1320C, A PUTATIVE MYOSIN LIGHT CHAIN OF *PLASMODIUM*

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Little is known regarding the nature and function of the myosin motors of *Plasmodium*. Analysis of the genome reveals that there are six myosin heavy chains but only the role of Myosin A, along with its cognate light chain partner, myosin A tail interacting protein (MTIP), has been well characterized in the process of parasite invasion. To date, only MTIP has been characterized as a *Plasmodium* myosin light chain. We have begun characterizing an annotated putative myosin light chain, however there is no biochemical evidence suggesting that the molecule functions in this manner. PFF1320c has been conserved within the primate infecting plasmodia, *P. vivax* and *P. knowlesi,* in addition to *P. falciparum*, yet this protein has been evolutionarily lost in the rodent parasites, *P. berghei*, *P. yoelii* and *P. chaubaudi*. Interestingly, in *P. falciparum* this protein appears essential due to our inability to disrupt the gene. However, upon expression of an HA tagged version of the gene at an ectopic site, we were able to knock out the gene. Preliminary studies have shown that this presumptive myosin light chain assembles in a high molecular weight complex indicative of formation of a myosin motor. Further studies are currently underway to identify the myosin heavy chain partner, determine the subcellular localization of this myosin motor and to characterize the biological role of this complex.

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A COMPARISON OF FOUR MOLECULAR ASSAYS FOR *PLASMODIUM* MALARIA PARASITES REVEALS FALSE POSITIVES AND FALSE NEGATIVES IN *ANOPHELES* VECTORS

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The detection of *Plasmodium* malaria parasites in *Anopheles* vectors is critical for understanding malarial transmission dynamics and for incriminating the vector species. Several PCR based methods have been developed for *Plasmodium* in human blood, but less so in the vector. The *18S rDNA* locus is the most commonly used molecular target although more multi-copy loci have recently been examined. We compared four *Plasmodium* specific molecular assays using field collected *Anopheles* mosquitoes from the Republic of Korea, and laboratory infected *An. stephensi*. The assays included: 1) a nested PCR technique targeting *18S rDNA* that has been previously used to test vectors, UNR/VIV; 2) a second commonly used *18S rDNA* assay, PLU/VIV; 3) a multi-copy nuclear marker, Pvr47; and 4) a nested PCR targeting the *Cytochrome B,* mitochondrial marker, *CytB*. Amplicon from PCR was sequenced and compared to known *Plasmodium spp*. in the *Plasmodium* Genome Resource (www.PlasmoDB.org). The specimens identified as positive, and the positivity rates, differed according to the assay. Two of the PCR assays, UNR/VIV and Pvr47*,* produced multiple banding patterns. DNA sequences for 3 of 4 PCR assays from the field collected specimens matched to non-*Plasmodium* organisms, which included the arthropod fungus, *Zoopthera sp.*. The nested *CytB* assay produced unequivocal results; detecting only one individual infected with *P. bergheii.* The *CytB* and UNR/VIV assays were then compared using *P. falciparum* infected *An. stephensi* incubated for different times post infection to measure sporozoite and oocyst detection rates. The *CytB* assay was more sensitive than the *18S rDNA* assay, detecting more infections of oocysts in the abdomen and of sporozoites in the salivary glands. We found that two commonly used primers for detecting *Plasmodium* targeting the *18S rDNA* region produces apparent false positives and negatives when applied to field-collected mosquito stage parasites. We recommend the use of the *CytB* assay to detect *Plasmodium spp*. in field studies, which aim to measure mosquito stage parasite rates.

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BLOOD FILMS AS DNA SOURCE FOR MOLECULAR DIAGNOSTIC OF MALARIA

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The use of molecular tools for malaria diagnostics has been spread successfully around the world; but its sensibility and specificity depends on the sample source and the quantity/quality of the DNA obtained. In order to explore other sources of parasite DNA, this study try to test if sufficient DNA could be successfully extracted from stained malaria blood films. One cultured blood sample with high parasitaemia (5%) was serially diluted in human whole blood, and 5 µL of each dilution was blotted on films in quadruplicates; then each film was stained with giemsa. Thus, from the four films of each parasitemia, two of them were extracted with Phenol-Chloroform and the other two with QIA amp DNA mini kit. The final elution step was made in 50 µL of buffer TE or 50uL of buffer AE respectively. As a positive control, the whole blood of each dilution was extracted with Phenol-Chloroform and QIA amp mini kit in duplicate. A total of 5 negative controls were used in order to detect cross contamination during the extraction process. The extracted DNA was amplified using a nested PCR method for *Plasmodium* species detection using 18S ribosomal gene as target. The DNA from blood films was successfully extracted with both, Phenol-Chloroform and QIA amp mini kit, methods. The detection limit of the nested PCR using DNA from stained blood films was 0.0016% of parasitemia compared to 0.00032% for the whole blood. None of the negatives controls amplified, showing that there were no cross contaminations during the extraction process. In conclusion blood films can be a good source of DNA to detect *Plasmodium* species by PCR without any problem of contamination, and the DNA from blood films combined with PCR could be used as a quality control for field diagnostic, or others genetics or molecular studies where other source of sample is difficult to obtain.

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UTILIZING DIRECT PATIENT SAMPLES FOR ANTIMALARIAL RESISTANCE GWAS IN *PLASMODIUM FALCIPARUM*

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Plasmodium falciparum malaria's rapid adaptation to new drugs allows it to remain one of the most devastating infectious diseases of humans. Understanding the genetic basis of these adaptations is critical to successful intervention. Association studies are an established tool for discovering the genetic mechanisms contributing to variation in drug responses. However, recent malaria-based GWAS have either had limited sample sizes due to the laborious nature of parasite culture adaptation or limited phenotype heritability due to the use of clinical phenotypes when avoiding culture adaptation. Here, we present a GWAS based on direct patient samples that utilizes *ex vivo* drug phenotypes and whole genome sequence of *P. falciparum*-enriched DNA using a hybrid-selection technique. This approach avoids both culture adaptation and clinical phenotypes and simplifies the process of performing a well-powered GWAS. We test 85 recently isolated parasites from Senegal against 8 antimalarial drugs_including amodiaquine, artemisinin, artesunate, dihydroartemisinin, chloroquine, pyrimethamine, mefloquine, and quinine. We adapt recent mixed-model GWAS tools, such as EMMA and GCTA, and selection tools, such as iHS and XP-EHH, to study the heritability of drug response phenotypes and identify known and novel loci associated with drug resistance at genome-wide significance. Additionally, we examine elements of population and genome structure and compare these to previously available sequence data from culture-adapted parasites. This demonstrates a highly scalable type of GWAS for antimalarial response, one that does not require time-intensive culture adaptation processes yet still utilizes an *ex vivo* drug phenotype that is less influenced by host or environmental factors than clinical phenotypes.

1202

CLINICAL AND PARASITOLOGICAL EVALUATION OF THE COMPARATIVE EFFICACY AND EFFECTIVENESS OF ARTEMETHER-LUMEFANTRINE, ARTESUNATE-AMODIAQUINE AND ARTESUNATE-AMODIAQUINE PLUS CHLORPHENIRAMINE IN NIGERIAN CHILDREN WITH ACUTE UNCOMPLICATED MALARIA

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Drug resistance has posed a serious threat to malaria chemotherapy in the past two decades. In order to curb this problem, WHO has recommended the use of artemisinin-based combination therapy (ACT) for the treatment of *falciparum* malaria. In Nigeria, artemether-lumefantrine (AL) and artesunate-amodiaquine (ASAQ) have been adopted as preferred options. However, ASAQ was only available as separate drugs that were co-administered until recently. Also chlorpheniramine has been reported to enhance the efficacy of amodiaquine. To encourage compliance, fixed dose combinations are recommended. Artemoclo™ (ATC), a fixed dose formulation of artesunate plus amodiaquine plus chlorpheniramine was evaluated for its comparative efficacy and effectiveness with AL and ASAQ for acute uncomplicated malaria in Nigerian children. Little is known about the therapeutic efficacy of these artemisinin combinations and the prevalence of molecular markers associated with antimalarial drug resistance. A total of 200 children with *Plasmodium falciparum* infection were recruited and randomized into three study groups (AL, ASAQ, and ATC). All patients were followed up for 42 days to study the clinical and parasitological responses according to the WHO protocol (2009). We assessed the polymorphism of the *pfcrt* and *pfmdr1*genes by direct sequencing method. Results will be presented here.

1203

THE EFFECT OF STORAGE CONDITIONS AND DNA EXTRACTION METHODS ON THE MOLECULAR ANALYSIS OF *PLASMODIA* FROM DRIED BLOOD SPOTS

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Extraction and amplification of parasite DNA from dried blood spots (DBS) has been widely used for the detection and genotyping of malaria parasites. However, there have been few systematic efforts to quantify the impact of DNA degradation on molecular assays. Using field samples spanning over a decade and well-characterized laboratory controls, we are evaluating the effects of duration of storage, storage conditions, and DNA extraction methods for DBS on 4 different PCR protocols: 1) nested PCR of 18S rDNA, 2) nested PCR of cytochrome B, 3) qPCR, and 4) single-round PCR of microsatellites. To date, we have completed evaluation of the effect of storage temperature and DNA extraction technique (saponin/Chelex vs. Qiagen spin column) for DBS containing known densities of laboratory-strain *P. falciparum* parasites (factors of 10 ranging from 1-10,000 parasites(p)/µl). Identically prepared samples were spotted on Whatman 903 filter paper, stored at -20C and ambient temperature for 2 years, than evaluated by PCR of 18S rDNA and 2 microsatellites. Samples stored at -20C visibly lysed more effectively during extraction. These samples were the only ones that were detected at 1p/µl, amplified more consistently (62.5% vs. 12.5% of samples) at ≤10p/µL by 18S PCR, and more robustly (2.4-4.7 fold higher peak intensities) by microsatellite PCR. A similar degree of increased sensitivity was seen comparing saponin/Chelex to spin column extraction yielding 62.5% vs. 12.5% positive respectively at ≤10p/µl by 18S PCR and a comparable increase in intensity (2.1-4.7 fold) by microsatellite PCR. The samples that were stored at -20C and Chelex extracted were detected at all parasite densities consistently (100% sensitivity) in 18S PCR and showed the highest relative concentration (4.7 fold higher compared to ambient column samples) of parasite DNA by microsatellite PCR. These data suggest that storage of DBS at -20C and extraction using the saponin/Chelex method provide greater sensitivity for detection of plasmodial DNA. More extensive analysis of field isolates is in process.

1204

CO-OCCURRENCE AND DISTRIBUTION OF EAST (L1014S) AND WEST (L1014F) AFRICAN KNOCKDOWN RESISTANCE MUTATIONS IN *ANOPHELES GAMBIAE S.L.* IN TANZANIA

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The use of insecticides has remained the principal component of malaria control. However, the development of resistance by mosquito vectors to insecticides recommended for IRS and/or LLINs would potentially affect the gains so far achieved in malaria control. The phenotypic data of the current national surveillance on malaria vectors susceptibility to insecticides clearly indicates that there is a notable increase in vector tolerance and/or resistance to pyrethroid insecticides in Tanzania. Two mutations in the sodium channel, *1014F* and *1014S* are known to cause target site resistance to pyrethroids and DDT. These mutations are often referred to as the West and East African knock down resistance (*kdr)* mutations, respectively. We screened and characterized the *kdr* mutations in *Anopheles gambiae s.l.* mosquitoes sampled from 14 sentinel districts in Tanzania. Following insecticide susceptibility tests of wild vector populations, presence and typing of the *kdr* genotypes were determined in *An. gambiae s.l* using allele-specific polymerase chain reaction tests. The East African *kdr* allele was found to be present in nine specimens out of 160 with a frequency ranging from 4% to 20%. The West African *kdr* allele in heterozygous form was found in 30 specimens out of 160 with a frequency ranging from 12% to 52%. The East African *kdr* mutation was detected in three sentinel districts of Ilala, Muleba and Handeni whereas the heterozygous form of West African *kdr* mutation was detected in Muleba, Babati, Mvomero and Ilala. This observation is supported by the phenotypic data which indicates increased levels of pyrethroid resistance in a number of vector populations sampled. This situation calls for an urgent implementation of national mitigation measures and rational resistance management strategies in the country, if the gains so far made in malaria control are to be sustained.

1205

CHARACTERIZATION OF INSECTICIDE RESISTANCES OF *CULEX QUINQUEFASCIATUS*, *ANOPHELES GAMBIAE*, *AEDES AEGYPTI* AND *AE. ALBOPICTUS* IN MAYOTTE (FRENCH COMOROS ISLAND)

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Several vector-borne diseases have affected and still affect Mayotte Island, the main ones being malaria, chikungunya, dengue, Rift Valley fever and filariasis. Since 1950s, insecticides are used against mosquitoes vectors of these diseases. Organochlorines (OC), organophosphates (OP), pyrethroids (PYR) and, more recently *Bti* (bacterial toxin), generate strong selection pressures on the field mosquitoes populations, but no data on the resistance status of mosquitoes vectors was available until now. Bioassays carried out on larval and adult field populations show strong resistance of *Culex quinquefasciatus* to temephos (OP), dieldrin (OC) and deltamethrin (PYR), and temephos resistance in *Anopheles gambiae*. However, *Aedes aegypti* and *Ae. albopictus* remain susceptible to these insecticides. Biochemical assays for various generalists detoxifying enzymes (metabolic resistance) showed a significantly higher esterase and glutanione-S-transferase activities, but a lower oxidase expression in the field population of *Cx. quinquefasciatus* compared to the susceptible reference strain. Esterases overactivity appears to be linked to the superlocus *Ester*. In fact, the *Ester*^{2} allele was present on the whole island. In *An. gambiae* an esterase overactivity was also observed. Other resistance mechanisms due to insecticide target site modifications were investigated. The *ace-1*^{R} allele, coding for an insensitive acetylcholinesterase (OP target), is present in the ten tested populations. The *kdr* mutation, associated with deltamethrin resistance, is almost fixed on the entire island in *Cx. quinquefasciatus*. The *rdl* mutation of the GABA receptor (dieldrin resistance) is also frequent. In *An. gambiae*, the *ace-1*^{R} allele was not present in the resistant population, so that temephos resistance in this species seems to be due only to overexpression of detoxification mechanisms. In a context of drastic reduction of the range of available insecticides in Mayotte, the presence of both metabolic and target site resistances in two of the main vectors of the island is particularly worrying. A better understanding of the resistance mechanisms involved is essential to implement more efficient and sustainable control strategies.

1206

WHAT IS THE VALUE OF 'INCREASED EFFICACY' FOR NEW VECTOR CONTROL TOOLS?

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Significant progress in malaria control in the past decade can be attributed largely to the massive scale-up of insecticide-based vector control interventions, such as long-lasting insecticidal nets (LLINs) or indoor residual spraying of insecticides (IRS). To date, few studies have assessed the impact of insecticide resistance on malaria control, although there are reports of the reduced efficacy of pyrethroid treated mosquito nets and pyrethroid based IRS from several countries across Africa. Monitoring is a critical component of vector control, and adequate resistance data should be collected and used to inform the choice of vector control tools. Clearly tools with the highest efficacy are preferable, particularly in areas of pyrethroid resistance. Knowledge of the type and level of resistance present in mosquito populations is critical for the interpretation and communication of field-derived data. Different laboratory and field-based methods used to assess the efficacy of vector control tools will be discussed, using recent examples from studies conducted across Africa. The use of models to explore the predicted community-level and health impact of new tools will also be discussed to highlight the importance of maintaining a high level of efficacy, especially in the presence of insecticide resistance.

1207

INDOOR RESIDUAL SPRAYING FOR DENGUE CONTROL: A FIELD EXPERIMENT IN PENANG, MALAYSIA

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Indoor residual spraying (IRS) is not used routinely for control of dengue vectors, and the method is not recommended by WHO for this purpose. However, there is evidence in the literature that where IRS was used for malaria control, *Aedes aegypti* populations were reduced or eliminated even though they were not the target species. The effectiveness of two residually-sprayed insecticides, Icon 10CS (pyrethroid; lambdacyhalothrin) and Actellic (organophosphate; pirimiphos methyl) was evaluated for control of dengue vectors in a small community of traditional housing in a

single location in Penang, Malaysia. Notably, a number of confirmed cases of dengue had been recorded in theis community in the weeks prior to the start of the study. The impact on peridomestic populations of *Aedes aegypti* and *Ae. albopictus* was measured using the standard *Stegomyia* and pupal indices. As impact on nuisance mosquitoes is important for any domestic treatment, *Culex quinquefasciatus* infestations were monitored using CDC miniature light traps, placed in a room overnight with human sleepers that were protected by untreated bednets. Shortly after the baseline study, the intervention began in January 2012. Initial analyses at the first follow-up survey demonstrated that indices fell immediately following treatment but that there were no differences between treatments. The trial is ongoing but final results will be presented and prospects for application of IRS for control of dengue and chikungunya vectors discussed.

1208

MATING COMPETITIVENESS OF RIDL® MOSQUITOES AGAINST WILD TYPE MOSQUITO STRAINS FROM DIFFERENT GLOBAL REGIONS

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The use of sterile insects as a control method for insects requires that laboratory-produced sterile males released in the field out-compete wild males in the insemination of wild females. The threshold for this competitiveness for male Mediterranean fruit flies sterilized by irradiation occurs when 20% of the females are successfully mated by sterile males while competing with an equal number of wild type (WT) males. A field trial using male *Aedes aegypti* mosquitoes carrying a dominant lethal (RIDL®) gene that was developed by Oxitec, has been proposed to be conducted in Key West, Florida. To assess the competitiveness of the RIDL males against *Ae. aegypti* males from Key West, MRFU is measuring the mating competitiveness in semi-field cages of RIDL males against *Ae. aegypti* males reared from eggs recently collected in Key West. This poster presents results from MFRU and a series of small cage and large cage, laboratory and semi-field mating competitiveness studies that have been performed on RIDL strains of *Ae. aegypti* and *Ae. albopictus* who competed against WT laboratory strains of these species in several laboratories around the world. These results clearly show that RIDL strains of mosquitoes can compete successfully with WT males in inseminating females. If RIDL males were equivalent to wild type, then, in these tests on average 50% of the wild females would have been mated by a RIDL male, while, in fact, we observed 48% of the mating was made by RIDL males. This is several times the minimum requirement for male competitiveness observed in a successful fruit fly control program, and extremely encouraging for the use of this control intervention with mosquitoes.

1209

ANOPHELES GAMBIAE SEMINAL TRANSGLUTAMINASE AND *IN VITRO* CROSSLINKING OF ITS NATIVE SUBSTRATE PLUGIN

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Male Anopheline mosquitoes coagulate their seminal fluids via cross-linking of a substrate, called Plugin, by a specific seminal transglutaminase. Successful transfer of the 'mating plug' produced by the crosslinking of Plugin is necessary for efficient sperm storage by females. Here, we report the expression and purification of both Plugin and its transglutaminase, and *in vitro* reconstitution of the crosslinking reaction. Full-length *Anopheles gambiae* seminal transglutaminase is monomeric and of mixed a/b character as predicted by sequence homology to other transglutaminases. The C-terminal domain of Plugin is monomeric and of pronounced a-helical character, but is highly extended in solution and aggregates in solution with increasing concentration. Ca2+-dependent crosslinking of Plugin by *A. gambiae* seminal transglutaminase occurs readily *in vitro*. Tryptic digestion and mass spectrometric analysis of crosslinked Plugin identified several crosslinking sites present in the majority of a-helices in the C-terminal domain. Inhibition of the seminal transglutaminase, thereby preventing formation of the mating plug, may represent a potential specific and effective chemosterilant for *A. gambiae*.

1210

NOVEL GENETIC MARKERS FOR INSECTICIDE RESISTANCE IN EAST AFRICAN *ANOPHELES GAMBIAE*

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Resistance to pyrethroid insecticides is increasing in East Africa and in some areas now approaches the high levels documented in many West African populations of *Anopheles gambiae s.s.*. Both metabolic and target site resistance-mechanisms have long been implicated for East African populations, but, apart from a single target site mutation (*kdr L1014S*) DNA polymorphisms associated with pyrethroid resistance in field populations have not been identified. To investigate the genetic determinants of resistance in *An. gambiae* from an area of high pyrethroid resistance near the Uganda-Kenyan border, we performed association studies for class I and II pyrethroids in two consecutive years. Families established directly from the wild that differed in resistance levels were screened using several methodologies ranging from low-density candidate SNP arrays to whole genome resequencing and expression microarrays. Few of the genes significantly overexpressed in resistant compared to susceptible families were from detoxification gene families, and levels of overexpression were low, suggesting that up-regulation of few key metabolic enzymes is not the major factor underpinning resistance. Using independent screening technologies, significantly associated SNPs from genotyping and resequencing were tested further for association in samples from other locations. Repeatable associations were obtained for several SNPs, most notably exonic variants in carboxylesterase and P450 genes. To our knowledge these are the first field-replicated markers for metabolic insecticide resistance in *An. gambiae*, and we have designed Taqman assays to facilitate routine diagnostic screening alongside *kdr* mutations.

1211

PHOTOLARVICIDAL DIET FORMULATIONS OF A PORPHYRIN DERIVATE TO POTENTIATE MALARIA VECTOR CONTROL

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Photolarvicidal approach based on the use of a photo-sensitizer associated to a suitable larval diet is a new strategy to potentiate the control of vector populations resistant to conventional insecticides. This study aimed to screen and to evaluate the photolarvicidal efficacy of two candidate diet formulations of a porphyrin photo-sensitizer derivate (C12 porphyrin) on *Anopheles gambiae* larvae in a semi-field situation in Burkina Faso. Powdered cat food pellet and pollen from several families of plants were used as candidate larval diets and were separately incubated in a series of C12 porphyrin solutions ranging from 3.110-3-2mM in order to obtain

final dried powders of sunlight-activatable diets. In a first dose-efficacy assay, an optimal effective dosage of each candidate diet was assessed using *An. gambiae* Kisumu strain maintained in two different samples of water. In a second assay, the efficacy of an optimal effective dosage of each candidate diet complex was offered to three different colonies of *An. gambiae* larvae maintained in natural larval breeding water originated from four different localities of western Burkina Faso. C12 porphyrin free food samples were used for control treatment. After overnight feeding in the darkness, larvae were exposed under natural light (sunlight) to be irradiated and their mortality was timely recorded. More than 79.79 ± 23.36 % of larval mortality was obtained whatever the C12 porphyrin-candidate diet. However, the mortality varied significantly according to the type of diet and the source of natural larval breeding water ($p<0.05$) with the highest larval mortality (100%) caused by the fine fraction of Cat food formulate. These results suggest that these sunlight-activatable C12 porphyrin-diet complexes should be considered as new environmental friendly tool which could be efficacy and cost effective against *An. gambiae* larvae. However more extended studies, need to be performed in different ecological set-ups to better validate the field efficacy of such formulations.

1212

BEHAVIOR OF MALARIA VECTORS AT THE BEDNET INTERFACE

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The management of insecticide resistance in African malaria vectors is entering a critical era and the need to identify novel approaches for controlling insecticide resistant populations is now more urgent than ever. Given our limited knowledge at present, any increase in understanding of vector behavior has the potential to improve the design of new tools or approaches for vector control as well as broadening our understanding of the modes of action and mechanisms of resistance to different insecticides. Here we report on the initial findings of a study to characterize behavior of *Anopheles gambiae s.s.* at key stages during host location and bloodfeeding, and to investigate how insecticides impinge on these basic responses. The nocturnal activity of free-flying individual or groups of mosquitoes in the laboratory and in the field is video recorded in the absence of visible light, and analyzed subsequently. Sequences of distinguishable behavioral events have been defined and quantified. As recordings demonstrate, these quantifiable events provide a standard against which variations in field populations can be evaluated. The impact on these behaviors of different insecticide treatments has been evaluated and results will be presented. Studies aim also to quantify the responses of pyrethroid resistant vector populations using these systems, and to develop more advanced systems to track and characterize the behavioral events at other stages in the process of hostseeking. Investigations are ongoing but the findings of work in progress that has been largely completed will be presented, and potential application to vector control will be discussed.

1213

EVALUATION OF NOVEL SYNTHETIC MOSQUITOCIDES FOR CONTROL OF *AEDES AEGYPTI* AND *ANOPHELES GAMBIAE*

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There are many diseases that are transmitted by arthropods, and mosquitoes vector a number of them, such as dengue fever transmitted by *Aedes aegypti* and malaria by *Anopheles gambiae*. Current control programs include indoor residual spraying, and the use of insecticide-treated nets. With the advent of mosquito resistance, there is an urgent need for new control measures, including new insecticides with novel modes of action. With this in mind, we have been screening unique compounds in both *An. gambiae* and *Ae. aegypti* in adult lethality bioassays and larval paralysis assays. In these studies, neurotoxicants, such as tetraethylammonium (TEA) and 4-aminopyridine were shown to be paralytic to larvae at low ppm levels. A number of novel catechols were used in the adult assays, which showed some promising results, and with equal kill on the resistant Akron strain of *An. gambiae*. It is our expectation that through novel chemistry and molecular design, we will find a compopund that will be effective in mosquitoes while at the same time have little effect on humans, providing a safe and effective class of new insecticide.

1214

PILOT TRIAL TO DEVELOP MOSQUITOCIDAL IMMUNITY IN CATTLE

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This project explored the possibility of producing mosquitocidal immunity in cattle as a way to reduce malaria transmission in regions where the local vectors are exophagic and zoophilic. Groups of 24 male Holstein calves were injected intramuscularly with either saline (control) or with bacteria-free *Anopheles stephensi* midgut homogenates taken from either unengorged or from blood-engorged mosquitoes. Two types of adjuvants (TiterMax and ISCOM) were used. Calves received 2 injections spaced 2 weeks apart. After the second immunization, nearly all calves immunized against midgut preparations had anti-midgut antibody titers >1:100,000. There was no noticeable difference in antibody response due to the different adjuvants. Preimmune sera and sera from saline-injected control calves had no anti-midgut antibodies. To test for mosquitocidal activity, ca. 150 mosquitoes each were loaded into feeding cassettes fashioned from Tupperware containers, duct tape and dental dam. Cassettes were strapped with plastic wrap to the shaved sides of calves for 20-30 minutes and then returned to the insectary where mosquitoes were released into cubic foot screened cages. Unfed mosquitoes were removed. Mosquitoes were checked daily and dead mosquitoes were counted and removed. Some (ca. 50) mosquitoes were transferred into smaller cages and allowed to oviposit. Eggs were counted and the average number of eggs laid per mosquito was calculated for each group. Vaccination with the various mosquito midgut preparations and adjuvants produced only a transitory and/or modest mosquitocidal effect in some of the immunized animals. None of the immunized calves reduced mosquito fecundity. The mosquitocidal effect in this trial was much less pronounced than the robust acaricidal effects seen in early anti-tick vaccine trials where calves were similarly immunized with homogenates of tick midguts. Neverthess, the fact that even a modest mosquitocidal effect was achieved indicates that efficacious mosquitocidal vaccines in livestock are possible.

1215

MALARIA VECTOR BIONOMICS AND HUMAN MALARIA INFECTION RATES IN NCHELENGE, ZAMBIA

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As part of the International Centers of Excellence in Malaria Research (ICEMR) project, mosquito collections and human malaria screenings were conducted from March to April 2012 in Nchelenge District in Luapula Province, Zambia. Nchelenge experiences hyperendemic malaria despite continued implementation of indoor residual spraying (IRS) and long-lasting insecticide nets (LLIN) as control measures. Center for

Disease Control light traps (CDC LT) and pyrethroid spray catch (PSC) collections from 58 households in Nchelenge revealed the presence of both *Anopheles gambiae* s.s. and *An. funestus* s.s. 1287 mosquitoes were collected and identified morphologically. *Anopheles* and Culicines made up approximately 70% and 30% of the total mosquito collection respectively. Female anophelines made up 53% of the total mosquito collection; *An. gambiae* s.s. and *An. funestus* s.s. represented 13% and 87% of the collection respectively. This indicates that *An. funestus* s.s. is the dominant malaria vector with some contribution from *An. gambiae* s.s. The abundance of *An. funestus s.s.* and the high human malaria infection rates in Nchelenge support the hypothesis of high anthropophilic behavior and high sporozoite infection rates in *An. funestus* s.s. Accordingly, it is predicted that the human biting index, human biting rate, and entomological inoculation rate for *An. funestus* s.s. is higher than that of *An. gambiae* s.s. The multiple blood feeding behavior of both malaria vectors will also be explored to identify heterogeneity in biting. The vector data in Nchelenge present unique opportunities to further our understanding of malaria transmission and the implications for malaria control in high-risk areas.

1216

ILLUMINATION PREFERENCE OF *ANOPHELES GAMBIAE* AND *AN. STEPHENSI* AT DAWN AND DUSK

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It seems likely that the preferences of female and male mosquitos regarding ambient illumination are intertwined with critical vector behaviors, including endophily, exophily, endophagy and exophagy. Nonetheless, we understand very little about the preferences of malaria vectors regarding ambient intensity of illumination. We have therefore developed and implemented a dual-choice illumination preference assay, a gradient illumination preference assay, and a wavelength preference assay using *Anopheles gambiae* and *An. stephensi*. We have begun with analysis of illumination preferences of both species at subjective dusk and dawn. Dual-choice and gradient assays reveal that female *An. gambiae* females exhibit a high preference index for low illumination in both arenas, whether introduced into the low- or high-illumination zones in these arenas. Male *An. gambiae* also exhibit a preference index favoring low and intermediate illumination, with a magnitude lower than that of female mosquitoes. We will present initial results regarding wavelength preference, spanning the visible spectrum, in *An. gambiae* and *An. stephensi*, and we will analyze the relationship between Zeitgeber time and illumination preferences in both species. These analyses will extend our understanding of illumination preferences in vector mosquitos, and set the stage for analysis of the dependence of these preferences on different rhodopsin family members and on circadian rhythms in vector mosquitos.

1217

THE EFFECT OF TEMPERATURE ON LIFE HISTORY TRAITS OF *CULEX* MOSQUITO POPULATIONS

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Culex mosquitoes,are primary vectors of *West Nile virus* and other pathogens in N. America. Evaluating the life history traits of *Culex* species and populations under variable temperatures is required to define the relationships between temperature, mosquito fitness and vectorial capacity. This is particularly important in light of changing global climates. Temperature is a significant abiotic factor for mosquitoes as it can directly affect development, mortality, morphology, and fecundity. Although the effects of temperature on pathogen proliferation for mosquito-borne agents are generally defined, a detailed understanding of the effects of temperature fluctuations on development of populations of *Culex* mosquitoes is lacking. Additionally,colonized and field populations can differ significantly in genetic and phenotypic diversity, as well as general physiology. Defining these differences is essential for interpreting studies of vector populations, which often rely on highly colonized populations to assess the effects of environmental conditions on vectorial capacity and fitness. We determined the effect of rearing temperatures including 16, 20, 24, 28, and 32^{o}C, on life history traits of colonized and field-derived populations of *Cx. pipiens, Cx. quinquefasciatus*, and *Cx. restuans* mosquitoes. Specifically, we measured temperature-dependent variation in life table characteristics such as development time, immature survival, adult survival, mosquito size, blood-feeding rates, and fecundity both among species and between colonized and field populations. Results demonstrate that all measured traits are significantly affected by temperature in all tested populations, yet also demonstrate that both species and population-specific effects significantly contribute to variation in these traits. In general, temperature and both immature and adult survival are negatively correlated, yet field populations of *Cx. pipiens* and *Cx. quinquefasciatus* survived longer than colony populations, particularly at lower temperatures. In addition, temperature was shown to significantly alter both body size and bloodfeeding rates and optimal temperatures for these traits differed among populations, particularly for *Cx. pipiens*. Taken together; our results demonstrate the significant effects of temperature on life-history traits and identify critical population and species-specific differences.

1218

GENETIC CONTROL OF MOSQUITOES: A WHIRLWIND TOUR OF GLOBAL ACTIVITIES

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Genetic control of mosquitoes has been envisioned, developed, and is now being implemented. Several approaches are currently at some stage of field implementation or are under development and trials are being considered. I will give a very rapid overview of the technologies and the current status of their degree of technology development, potency and field implementation. While the evaluation is obviously subjective, the overview will provide the uninformed with their current status in a nutshell. The overview will cover all genetic approaches as defined by "Dissemination, by mating and inheritance, of factors that reduce pest damage." These include transgenic (RIDL, HEGs, Refractory) and non-transgenic approaches (*Wolbachia* and radiation-sterilized) mosquitoes. "Technology development" indicates my evaluation of how far the technology has matured toward a workable tool. "Potency" will be evaluated in terms of the versatility of the technology, the likelihood that the cost:benefit favors widespread implementation and effectiveness in developing countries and the intrinsic durability of the intervention. "Implementation" is an assessment of whether field activities are actually occurring and in a way that represents a full-blown public health intervention.

1219

RAPID COMPARATIVE EVALUATION OF ANOPHELINE SAMPLING METHODS IN THREE LOCALITIES IN INDONESIA

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Effective sampling techniques are necessary to monitor vector populations and evaluate the effectiveness of vector control interventions. Most methods for monitoring malaria vectors have been developed for trapping anthropophilic, indoor biting African mosquitoes and may not be effective

in areas with diverse, outdoor biting vectors such as those in Indonesia. In this study, eight different collection methods were tested in three different locations in Indonesia representing different malaria transmission zones: a low transmission field site in Purworejo, Central Java; a medium transmission field site in Lampung, Sumatra; and a high transmission area in South Halmahera. Indoor and outdoor human landing catches, CDC light traps, resting pots and boxes, as well as several exposure free tents: the Ifakara Tent Trap model C, large and small human-baited tents were tested for both total number of anophelines per night and species captured. A goat-baited tent was also tested in one of the three locations. Traps were tested in a 3 x 3 Latin-square design and mean anopheline catches per trap night were compared using ANOVA. Samples were morphologically identified and screened by cs ELISA and sporozoite diagnostic PCR and species identifications were molecularly confirmed by sequencing the ribosomal DNA ITS2 region. In Lampung, a total of 2362 anophelines were collected over 16 catch nights, 5 of which were found to be positive for *P. falciparum* and 57 samples positive for *P. vivax*. In Purworejo, 286 total anophelines were collected over 16 catch nights, with 2 samples positive for *P. vivax*. In Halmahera, 63 anophelines were collected over 8 nights, with no *Plasmodium* positives. We conclude that outdoor human landing collection remains the most effective method for collecting malaria vectors in Lampung and Purworejo, and a goat-baited tent (not tested at the other two sites) most effective in Halmahera. Effectiveness of each trap on the 10 different *Anopheles* species captured over the three sites will be presented.

1220

LARVAL SOURCE MANAGEMENT AND MOSQUITO-BORNE PATHOGEN TRANSMISSION

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Mathematical models of malaria transmission have influenced strategic decisions about malaria control since the time of Ronald Ross, when larval source management (LSM) was the dominant mode of malaria prevention. Following the success of early trials using indoor residual spraying (IRS) with DDT, George Macdonald showed that transmission was highly sensitive to adult mosquito mortality rates, which reinforced the prevailing notion at the time that DDT alone was sufficient to eradicate malaria. Recent policy recommendations for vector control did not include LSM, based once again on Macdonald's analysis. Recent empirical evidence demonstrates that, in some circumstances, LSM can be as effective or as cost-effective as IRS or insecticide-treated bed nets. A recent review demonstrated that the mathematical theory underpinning control of terrestrial adult and aquatic immature mosquito populations have not been developed to the same extent, however. No simple theory exists for LSM that is comparable to Macdonald's analysis of adult mortality. Here, we present a simple model describing mosquito population dynamics, update Macdonald's original analysis, and establish basic theory for LSM. One of the key features of our model is that aquatic mosquito populations are distributed among distinct aquatic habitats. With density dependence in these structured aquatic habitats, the effect sizes of LSM respond in a non-linear way with the proportion of treated aquatic habitats. In other words, removing 50% of larval productivity reduces mosquito population density by more than 75%. We discuss the factors, such as spatial distribution of aquatic habitat, variation in productivity among habitats, and mosquito egg-laying behavior, that affect the efficacy of LSM.

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PREDICTING *ANOPHELES GAMBIAE* LARVAL HABITAT LOCATIONS IN LOWLAND, WESTERN KENYA

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Malaria risk to humans is heterogeneous at the community scale. The locations of people's houses relative to the locations of larval *Anopheles* habitats have been shown to influence the spatial distribution of malaria infections in certain landscapes. In lowland, western Kenya, *Anopheles gambiae* larval habitats are numerous and widespread, and the relationship between larval habitat distribution and malaria transmission is less clear. Accurately predicting the locations of larval habitats will allow us to test this relationship across a large area. We used geographic information over a 10 by 10 km landscape in Asembo, Kenya to model the locations of *An. gambiae* larval habitats. Thirty-one sample quadrats (500 by 500 m) were exhaustively surveyed, and all potential larval habitats were georeferenced with GPS. A raster data model at 20 m spatial resolution was built around five geographic variables for the study area: elevation, distance to the nearest stream, land cover/land use categories, and two indices of topography related to pooling water (slope:area ratio and locally low index). These variables were tested for their ability to predict habitat locations using logistic regression modeling. Preliminary results suggest the importance of all five variables in predicting the locations of *An. gambiae* larval habitats. Habitats were more likely to be found in areas where the slope:area ratio and locally low index values predicted pooling water. They were also more likely closer to streams and in agricultural land. Future work includes using predicted *An. gambiae* larval habitat locations to explain heterogeneity in adult *An. gambiae* spatial distribution.

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ESTIMATING LONG-TERM *AEDES AEGYPTI* ABUNDANCE IN IQUITOS, PERU USING A NOVEL, SPATIALLY-EXPLICIT SMOOTHING METHOD

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Understanding patterns of spatial and temporal variation in *Aedes aegypti* abundance is crucial for predicting dengue incidence and modeling virus transmission dynamics. Since 1999, over 150,000 entomological surveys were conducted within individual homes throughout urban Iquitos, Peru, using a standardized collection procedure. Based on data from a 1 year period between interventions (n=13,109 surveys), we estimated a mean adult mosquito abundance of 0.577 per house (negative binomial, size = 0.166). Adult mosquito counts were highly over-dispersed and varied greatly across space ($p<0.05$). Accurately estimating adult mosquito abundance over long time periods is complicated, however, by spatiotemporal heterogeneity in the mosquito environment, limitations on mosquito capture efficiency, and the coverage and timing of interventions. To account for variation over the full 13 years of data, we thus develop a non-parametric smoothing approach that creates a single time-series to describe city-wide mosquito abundance as well as a surface that describes spatial differences across the urban landscape. Since we avoid restrictive seasonality assumptions we are able to easily compensate for intervention efforts as well as discern inter-annual variation in the timing of peak mosquito abundance. We are also able to identify, on a small scale, locations that systematically over- or under-produce relative to the rest of

the city. Future work will combine our time-series and surface describing mosquito abundance with spatiotemporally explicit dengue infection data to better understand and predict the patterns of dengue outbreaks.

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MOSQUITOES *(DIPTERA:CULICIDAE)* FROM THE NORTHERN PERUVIAN AMAZON

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An entomological survey was conducted in four villages (Lagunas, Santa Cruz, Saramiriza and Puerto America) 635 km southwest of Iquitos in the northern Peruvian Amazon Basin, to determine mosquito population demographics and predict human risk of exposure to mosquito-borne diseases in this region. Collections were made using CDC light traps placed in extradomiciliary areas (4-20 traps/site, 100 m away from houses) from 1800-0600 hours; protected human bait situated in peridomiciliary areas from 1800-2100 hours; and backpack aspirators inside houses (20 min/house, 50 houses/site) from 0800-1245 hours. A total of 22,513 mosquitoes were identified, belonging to two sub-families, Culicinae and Anophelinae, 12 genera, 14 sub-genera and 48 species. *Culex* and *Mansonia* spp. accounted for 57% and 18%, respectively, of the total mosquitoes collected by all methods combined. CDC light trap captures contained the largest mosquito number and the greatest species diversity (16,947 mosquitoes, 44 species). Human bait (3,888 mosquitoes, 27 species) and backpack aspirators (1,678 mosquitoes, 25 species) captures were smaller. Trap collections reflect typical genera bias: CDC light traps contained 68% *Culex* spp. and 9% *Ochlerotatus* spp., while human bait collections consisted of 75% *Mansonia* spp., and backpack aspirator collections contained 68% *Culex* spp. and 29% *Mansonia* spp. The average hourly collection rates (mosquitoes/hour, m/h) differed between collection methods: 118 m/h for human bait, 88 m/h for CDC light traps, and 15 m/h for backpack aspirators. Identified Anophelinae belong to the subgenera *Anopheles, Nyssorhynchus* and *Stethomyia*. *An. oswaldoi, An. benarrochi and An. mattogrossensis* had the highest density. The Shannon-Weaver diversity index (H) ranged from 0.83 (Santa Cruz) to 1.04 (Lagunas), indicating a potentially high mosquito diversity in the northern Peruvian Amazon, where *Culex* spp. and *Mansonia* spp. are the most abundant. Several of the mosquito species collected in this study are of interest because of their potential role as vectors of arbovirus and parasites in the Peruvian Amazon Basin.

1224

DEMONSTRATION OF A PUSH-PULL STRATEGY FOR DENGUE VECTOR CONTROL: OBSERVATIONS FROM LOCAL THAI HOUSES

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A pilot demonstration trial for a repellent focused push-pull strategy was conducted at local homes in two Thai villages. The purpose of the trial was to validate findings from experimental hut studies that have been under evaluation for the last 4 years in both Thailand and Peru and to determine the challenges in implementing the strategy under a "real-life" scenario. The goal of the push-pull system is to reduce densities of the dengue mosquito vector, *Aedes aegypti,* inside homes. The strategy has two components: 1) the "push" which involves the placement of a spatial repellent treatment inside homes with the intent to deter the mosquitoes from entering and 2) the "pull" which involves the placement of a mosquito trap outside the home to capture repelled mosquitoes in the peridomestic environment. Combined, the approach would reduce human-vector contact thereby decrease the probability of virus transmission. While experimental hut studies at both research sites have demonstrated efficacy of the push-pull strategy to impact densities of adult mosquitoes entering treated huts as compared to matched controls, and when either tool is used separately, it was necessary to test the strategy at local homes to measure expected changes in efficacy from experimental to natural conditions and define the impact on adult *Ae. aegypti* inside houses where people reside. Such information is vital to provide evidence of effectiveness and applicability in local settings for future strategy development and to establish entomological correlates of impact to drive translational research.

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POTENTIAL FOR DENGUE VIRUS TRANSMISSION IN SOUTH TEXAS

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Semiarid subtropical South Texas is a unique ecoregion in the United States. The geographic and socio-economic conditions of this region may increase the risk of endemic dengue transmission. We conducted an initial study in summer 2010, examining factors that may influence dengue transmission. We studied two potential dengue vectors, *Aedes albopictus* and *Ae. aegypti*, found in the region in the summer of 2010 to identify distribution patterns and relative abundance. We used, oviposition traps to collect eggs from these container breeders and hatched the eggs to identify species. We also surveyed local residents at key field sites to assess behavioral factors influencing dengue transmission. Research questions incorporated knowledge of mosquitoes, frequency of outdoor activity, and awareness of mosquito activity. Our results show that both *Ae. aegypti* and *Ae. albopictus* are present in South Texas, despite a drought that was concurrent to the research study. In addition, human behavior and knowledge varied widely, with a range of survey results showing a population that may be at increased risk due to lack of awareness or increased risk of exposure to the potential disease vectors. Our preliminary study suggests that South Texas may be at increased risk of endemic dengue transmission, and further studies are warranted.

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ANOPHELES ABUNDANCE AND BLOOD FEEDING PREFERENCES IN SOUTH TEXAS

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Anopheles mosquitoes are the primary vectors for the parasitic disease malaria. Endemic malaria has been eradicated from the United States; however there is still a low risk for endemic malaria transmission in Mexico. South Texas may remain at risk for malaria importation due to a shared border with Mexico. To understand the risk of endemic malaria transmission in the Lower Rio Grande, South Texas, we studied the distribution and abundance of the malaria vectors in this region, *An. quadrimaculatus* and *An. pseudopunctipennis*. In addition to studying species composition, abundance, and distribution, we examined blood feeding preferences in order to better assess malaria transmission potential. We conducted a three-month survey of the mosquitoes in the Lower Rio Grande Valley from late June through mid-September using light traps and resting boxes. Four trapping sites along the Rio Grande River from Mission, Texas to Brownsville, Texas were studied. *Anopheles* mosquitoes collected weekly were identified to species. Blood fed *Anopheles* were analyzed with real time PCR to identify blood meal sources with assays designed to distinguish mammal blood meals from other vertebrates. We collected a total of 122 *Anopheles* mosquitoes from three of the four sites, 23 of which had a blood meal prior to capture. Sixteen out of the 23 blood fed *Anopheles* mosquitoes were identified

as feeding on mammalian blood. The presence of *Anopheles* mosquitoes capable of transmitting malaria in the Lower Rio Grande Valley may indicate there is a potential risk of endemic malaria transmission in South Texas.

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BLOOD MEAL IDENTIFICATION USING REAL TIME POLYMERASE CHAIN REACTION

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To control or eradicate vector-borne disease, a detailed understanding of the disease transmission dynamics is required. This requires critical assessment of feeding behaviors, preferences, and strategies of disease vectors, including frequency of feeding on human hosts. Current methods of identifying host feeding preferences include gene sequencing, classical PCR, serological techniques, and ELISA assays. One technique seldom developed is utilizing real-time PCR methods. Real-time PCR techniques allows for the rapid identification of blood meal sources, increased sensitivity to small amounts of blood, and quantification of relative proportions of blood from different hosts. Our new method discriminates between human blood meals, other mammalian blood meals and non-mammalian blood meals. Using comparative genomics of human, other mammals and other vertebrates, regions encoding micro-RNA (miRNA) were identified. MiRNAs are small, less than 120 nucleotides, conserved loci that occur in all organisms. Several miRNAs were screened for their specificity using classical PCR and two were chosen to use for the real-time PCR assay; one identifying humans and one identifying all mammals. The assays were tested for specificity using human, cow, guinea pig, horse, rabbit and dog DNA as positive controls, Asian tiger mosquito, chicken and duck DNA as negative controls. The assays were then optimized on a Cepheid Smart Cycler and Illumina Eco Real Time PCR system using Taqman chemistry. By using a FAM and CalOrange fluorescent probe for each assay, the two can be run as one as a duoplex reaction. Our method provides a rapid, detailed analysis of blood feeding behavior and identifies preferences in host type. Additional assays for avian detection and an internal mosquito control may be developed as well.

1228

EVALUATION OF POINT MUTATIONS IN THE ATTENUATION OF YELLOW FEVER VIRUS

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Whilst the 17D vaccine has been used extensively for the control of yellow fever virus (YFV), the roles of the specific mutations in its attenuation in both mammals and mosquitoes remain unknown. Previous research has demonstrated the importance of the envelope protein domain III (ED3) in governing the attenuation process and the virulence amongst various flaviviruses. With the cDNA infectious clones of the 17D strain, Asibi strain and chimeric viruses containing the structural region of Asibi strain on the 17D strain backbone, we evaluated the roles of two mutations located in ED3, M299I and T380R, for the infection and dissemination in *Aedes aegypti*. Detection of viruses in the bodies, heads, legs and wings of mosquitoes was based on $TCID_{50}$ titration. Infection was reported based on the detection of viruses in either the whole body or any tissues above. Dissemination was confirmed by the detection of viruses in the heads, wings or legs. The effects of the specific mutations on infection and dissemination in mosquitoes will be discussed.

1229

MODELING THE ECOLOGY OF GENERALIST PATHOGENS IN MULTIPLE MOSQUITO VECTORS

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Models of multi-host, multi-vector mosquito-borne pathogen transmission have been underrepresented considering the issues faced in dealing with pathogens such as sylvatic Dengue virus, Rift Valley Fever and West Nile Virus that are transmitted among several host species by several mosquito vectors. Mathematical models that make different assumptions about the distribution of bites among hosts lead to differing pathogen transmission dynamics. Here we review the multi-host, multi-vector pathogen literature, and present theory unifying and summarizing key aspects of transmission and compare the behavior of major classes of models. We focus on three main areas: tradeoffs between vector preferences and host availability, the role of frequency versus density dependence for vectors and hosts and the roles of host movement and spatial distributions on vector feeding. We mechanistically derive model formulas, present simulations and data informing each of the three areas. Models presented encompass a wide range of ecological scenarios including populations of different species of hosts, meta-populations of a single species separated spatially with or without movement between patches, or heterogeneous biting of a vector on single or multiple host species. Multi-host, multi-vector models encompass pathogens causing significant disease burden. Zooprophylaxis for malaria and the dynamics of pathogens in complex multi-host and multi-vector environments can be further elucidated through the development of theory based on mosquito feeding behavior and ecology and its interactions with host behavior and ecology that lead to patterns of blood feeding. Detailed understanding of transmission cycles of these pathogens could have considerable and direct impact on those living in and around areas with natural zoonotic cycles and for those connected to those populations through human or animal movement.

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DEFORESTATION AND URBANIZATION DRIVE THE INVASION AND TRANSMISSION OF A VECTOR BORNE DISEASE

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Anthropogenic land use has altered much of the planet's surface. This has changed host and vector communities and altered climates. The impact of these changes on the transmission of zoonotic pathogens is difficult to predict because of the numerous interactions among hosts, vectors, and pathogens. We examined detailed aspects of transmission ecology for a vector-borne pathogen, West Nile virus, across a land use gradient from intact forest to highly urbanized cities. We found a strong gradient in transmission potential with viral transmission absent in intact forests and intense yearly transmission in urban areas. This pattern was driven by a combination of changes in vector abundance and species composition, vector feeding patterns, and host community composition, with microclimate playing a smaller role at this spatial scale. These results highlight the key role of changes in the animal community in response to land use change that play an ever increasing role in human health.

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A MATHEMATICAL MODEL FOR *ANOPHELES FARAUTI* ECOLOGY AND MALARIA DYNAMICS IN HALETA VILLAGE, SOLOMON ISLANDS

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Malaria control programs have a variety of available deployable tools, including multiple options for vector control. The potential impact of different vector control tools depends on the local mix of vector species, their ecologies and their feeding behaviors. The EMOD model represents mosquito ecology and feeding dynamics in the presence of interventions in order to study the impact of vector control and other malaria campaign tools upon malaria transmission. The basic model is adapted to study vector ecology and malaria transmission in Haleta village in the Solomon Islands and the local vector *Anopheles farauti*. A new sub-module within the malaria transmission model is created for *An. farauti* ecology and larval habitat, which is primarily a brackish lagoon near the village. Current and historical data for vector feeding behavior are utilized to study the predicted impact of bednets and other vector control tools. Human blood index, vector feeding and resting locations, and timing of feeding are examined for sensitivities upon the impacts of vector control. Given the high rate of outdoor feeding early in the evening and outdoor resting post blood meal, the impact of bednets as a vector control tool is predicted to be limited. The impacts of other vector control tools are simulated to examine possible effects both in combination with and in place of bednets. The local study site, its vector data, and the resulting model outputs demonstrate the importance of understanding vector ecology and behavior in designing locally tailored vector control components of malaria control and elimination campaigns. The VECNet Consortium brings together vector ecologists, tool developers, mathematical modelers, and public health professionals to study these data and their implications.

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MOSQUITO MIDGUT MICROBIOTA PARTICIPATES IN THE CONTROL OF DENGUE VIRUS AND *PLASMODIUM FALCIPARUM* IN HOST VECTOR

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The resident microbiota of insect vectors can impede transmission of human pathogens. Recent studies have highlighted the capacity of endogenous bacteria to decrease viral and parasitic infections in mosquito and tsetse fly vectors by activating their immune responses or directly inhibiting pathogen development. These microbes may prove effective agents for manipulating the vector competence for malaria and other important human pathogens, as well as representing promising sources of anti-pathogen therapeutic natural products. Our purpose was to collect field mosquito and study the natural midgut microbiota allocated in them. We have identified a variety of microbes of the mosquito midgut microflora with potent *in vivo* and *in vitro* activities against the dengue virus and different stages of the malaria parasite *Plasmodium*. Indeed a couple of them show activity against both pathogenic agents. The potential of these microbes for disease control will be discussed.

1233

MOLECULAR AND MORPHOMETRIC ASSESSMENT OF *ANOPHELES ALBITARSIS S.L.* FROM COLOMBIA

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Recent barcode analysis revealed eight species and one lineage within the Albitarsis Complex. Adult females from this complex cannot be differentiated by morphology, but accurate species identification is important for effective and targeted vector control programs. In this study, geometric morphometric (GM) analysis of the wing and *COI* barcode analysis were used to: 1) establish the taxonomic status of *Anopheles albitarsis s.l.* collected in localities of five departments of Colombia and 2) evaluate the potential of GM to separate species of the complex. Preliminary, *a priori* species assignation using DNA barcodes determined the presence of two species, *An. albitarsis* I distributed in north and central Colombia and *An. albitarsis* F found in eastern Colombia. GM analysis of thirteen wing landmarks of type I (venation intersections) of 98 specimens and interspecific wing size comparison showed significant differences between members of both species. *An. albitarsis* I had the smallest wing (average centroid size=3.187 mm±0.019 SE) and *An. albitarsis* F the largest (average centroid size=3.337 mm±0.043 SE). Allometric effect on shape variation was no significant (p>0.05). The interspecific wing shape analysis was non discriminant and non-informative (p=0.144). Subtle differences in wing size could be explained by several environmental variables such as elevation, temperature and food sources in breeding sites. While wing shape was not useful for species delimitation due to morphological overlap, DNA barcoding supported the presence of two species remaining as the method of election for discriminating among these species.

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EXPERIMENTAL STUDIES OF THE DISPERSION OF FEMALES OF TWO *ANOPHELES* SPECIES AROUND INSECTICIDE-TREATED AND UNTREATED BED NETS: IMPLICATIONS FOR ASSESSING THE SIGNIFICANCE OF PHYSICAL DAMAGE TO BED NETS

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Arrays of sticky panels were used to sample released not-previously-blood fed female mosquitoes flying near and landing on sectors of bed nets with and without a person inside. Experiments were done in an environmentally-controlled room with insectary-reared *Anopheles gambiae* G3 or *An. albimanus*, with insecticide-treated (Permanet 2.0®) or untreated bed nets (180x150x130cm) and under four combinations of temperature and humidity (warm-humid, warm-dry, cool-humid, cool-dry). The presence of a person in both treated and untreated bed nets significantly increased total net catch for all temperature-humidity combinations for both mosquito species. For all cases when a host was present, except the case of *An. gambiae* G3 in warm-humid conditions, catches were larger on the sticky panels on the net roof than on panels on the sides and ends. Total catch on treated net panels was somewhat lower than on untreated net panels due to the insecticidal effect but mosquito dispersion patterns did not differ between net types. Results indicate that mosquito pressure varies with location on the bed net depending on mosquito species and ambient conditions. The implications of these findings for situation-appropriate assessment of bed nets that have sustained physical damage in the form of holes, rips and tears is discussed.

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MALARIA VECTOR MOSQUITOES IN NINE KOREAN ARMY-BASE CAMPS NEAR DEMILITARIZED ZONE IN THE REPUBLIC OF KOREA, 2011

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Population density and *vivax* infection rates of *Anopheles* mosquitoes from 27 points (3 points per one base camp; mess hall, guard point and soldier's shelter) in nine Korean army base camps (composed of three General outpost (GOP), three Forward Edge of Battle Area (FEBA) 1 and three FEBA2) near Demilitarized zone (DMZ) in Paju-si and Yeoncheon-gun, Gyeonggi-do, Republic of Korea (ROK) were monitored with black light traps from May to October for supporting basic information for vector control. Members of *Anopheles* mosquitoes were identified to species and were tested for *vivax* infection by Polymerase chain reaction (PCR) with genomic DNA. Of 4,282 mosquitoes, a population density rate of *Anopheles* mosquitoes was over 50% and *An. kleini* (69.5%) was predominant among eight *Anopheles* mosquito species, followed by *An. pullus* (17.3%), *An. belenrae* (4.9%), *An. sineroides* (4.2%), *An. sinensis* (2.7%) and *An. lesteri* (1.9%). No *An. koreicus* and *An. lindesayi* were collected. *An. kleini* exhibited the highest *vivax* infection rates, followed by *An*. *pullus*, *An*. *belenrae*, *An*. *sineroides*, *An*. *sinensis* and *An*. *lesteri*. The *vivax* infection rates of the vector mosquitoes steadily increased from May to October and was the highest in July. Order from highest to lowest mean population density of *Anopheles* mosquitoes was the GOP inside DMZ, the FEBA 1 and FEBA 2 outside DMZ. The mean population density of *Anopheles* mosquitoes in the mess hall in each base camp was higher than that in guard point and soldier's shelter. In our study, *vivax* infected vector mosquitoes in the base camps were collected from May to October and soldiers in the base camps were always exposed to *vivax* malaria during the mosquito season. In the ROK, malaria cases in Korea army base camps near DMZ were over 40% of total malaria cases every year. Malaria management in Korea army is very important to eliminate malaria in the ROK. The effective and accurate vector control should be need from May to October for malaria management in Korea army base camps. Population density and *vivax* infection of vector mosquitoes in Korea army base camps near DMZ should be continuously performed and extend monitoring areas.

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MOSQUITO SPECIES COMPOSITION AND *PLASMODIUM VIVAX* INFECTION RATES IN KOREA ARMY BASE-CAMPS NEAR THE DEMILITARIZED ZONE IN THE REPUBLIC OF KOREA, 2011

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Vivax malaria is a significant military and civilian health threat in the north of the Republic of Korea (ROK). Vector mosquito collections were performed in the Korea two army base-camps in Paju-si near the Demilitarized zone (DMZ) using black light traps in 2011. The DMZ is the northernmost point of the ROK and is located close to the Democratic People's Republic of Korea (DPRK). *Anopheles* spp. were assayed by PCR, to identify the species, and screened for sporozoites of *Plasmodium vivax*. Of 4,354 female *Anopheles* mosquitoes collected, *An. kleini* was the most abundant, followed by *An. pullus*, *An. belenrae*, *An. sinensis*, *An. sineroides*, and *An. lesteri*. *Anopheles kleini*, *An*. *pullus* and *An*. *sineroides* demonstrated the highest population density in June and *An*. *belenrae*, *An*. *lesteri* and *An*. *sinensis* in August. No non-Hyrcanus group species, *An. lindesayi japonicus* and *An. koreicus*, were collected. The value of the total maximum likelihood estimation (TMLE) [estimated number of positive mosquitoes/1,000] for *P. vivax* value of *An*. *lesteri* was the highest, followed by *An*. *sineroides*, *An*. *belenrae*, *An*. *sinensis*, *An*. *pullus* and *An*. *kleini*. The seasonal maximum likelihood estimation (SMLE) values were different depending on *Anopheles* species. *Anopheles belenrae*, *An*. *pullus* and *An*. *sineroides* showed the highest SMLE values in July and *An*. *lesteri* and *An*. *sinensis* exhibited the highest SMLE values in September. *Anopheles kleini* showed the highest SMLE values during August. This is the first report of an *An*. *sineroides* positive for *P. vivax* in the ROK. The results demonstrate new information of *An*. *sineroides* as a potential vector and extend our knowledge of the distribution and a potential role in malaria transmission of vector mosquitoes at Korea army base-camps in areas previously considered to be at a high risk in the ROK for contraction *vivax* malaria.

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SEQUENCE ANALYSIS OF THE INFLUENZA A (H1N1) PDM09 VIRUS HAEMAGGLUTININ (HA) GENE CIRCULATING AMONG INFLUENZA-LIKE ILLNESS (ILI) PATIENTS IN EGYPT

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Influenza viruses and influenza A/H1N1 pdm09 in particular have been associated with a range of clinical presentations from mild to severe and/or fatal disease. Specific genetic mutations in the HA gene of A/H1N1 pdm09 are thought to be associated with virulence and disease outcome. pH1N1 was first identified in Egypt in June 2009, from patients with recent travel history. In this study we analyzed the HA gene of A/H1N1 pdm09 virus circulating among ILI patients in Egypt during the peak season to monitor mutations that could potentially lead to increased virulence. 592 oropharyngeal swabs from ILI patients were collected from November 2009 -January 2010, from eight sentinel sites distributed throughout Egypt, then screened by real-time RT-PCR to detect A/H1N1 pdm09. The virus was isolated and representative isolates were chosen for HA sequencing (Sanger) followed by phylogenetic analysis (MEGA4). Influenza A/H1N1 pdm09 was identified in 27% (n=161) of ILI cases. The HA gene of the 42 representative isolates had 99.1%-99.5% nucleotide identity and 2-8 AA differences from the vaccine strain (A/California/07/2009). All analyzed isolates had the P83S mutation; other mutations including S203T (98%), D222E (71%), P297S (71%) and I321V (86%) were also present. All isolates having the D222E also revealed the P297S mutation; however D222G/N mutations reported to be associated with severe cases were not found. All D222E containing viruses clustered together in the phylogenetic tree. Parallel mutations at certain sites in the majority of the viruses from Egypt may be indicative of active positive selection. Further studies are needed to understand the evolution of the D222E mutation and its effect on receptor binding specificity, antigenicity, transmissibility and virulence of Influenza A/H1N1.

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ETIOLOGY OF FEVER AND ACUTE RESPIRATORY ILLNESS IN NEWBORNS, CHILDREN, AND ADULTS IN A RURAL AREA OF PAKISTAN - A PROSPECTIVE, COMMUNITY BASED SURVEILLANCE STUDY

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Acute respiratory infections (ARI) account for 24% of all under-5 child deaths in Pakistan, translating to an estimated 90,000 deaths annually. There have been no comprehensive studies of the etiology of severe pneumonia in Pakistan since the 1980s. The goal of our study is to determine the etiology of ARI and febrile illness in different age groups in Pakistan, using cutting edge diagnostic modalities including real-time PCR and automated culture methods. This is a longitudinal cohort observational study of newborns, young children, and their adult household members with fortnightly surveillance throughout the duration

of this three year study. A WHO case definition of severe pneumonia is employed, and cases of febrile illness are identified using temperature ≥ 101.3ºF (for newborns) or ≥ 100.4 ºF (for young children and adults). Participants are evaluated by mobile medical teams to obtain appropriate samples and for medical treatment of the observed illnesses. Samples include nasopharyngeal swabs for workup of ARI by PCR identification of bacterial and viral etiologies and blood cultures and malaria ICT for workup of febrile illnesses. In the initial 6 month period of this study, 94cases of severe pneumonia in newborns with or without fever and 8 cases of newborns having fever ≥ 101.3ºF only have been identified. In children 5 to 14 years of age, 5 cases of fever ≥ 100.4 ºF only and 7 cases of ARI without pneumonia have been identified. In adults, there have been 8 cases of fever ≥ 100.4 ºF with influenza-like symptoms and 7 cases of ARI without fever. To date, 35 nasopharyngeal swab samples out of 165 collected from newborns have been analyzed using Luminex assay RVP Fast kit. Of these samples, 16 samples were positive for enterovirus / rhinovirus, 2 were positive for RSV, 5 were positive for PIV type III/IV, and 12 cases were negative for the pathogens tested. Blood cultures were positive in 2 cases, with one case of Campylobacter and E.coli each. Malaria ICT was positive in 2 cases showing combined *Plasmodium falciparum* and *P. vivax* infection. We are currently in the first year of this three year surveillance study. The results of this study will promote establishment of empiric treatment algorithms for ARI and febrile illness in Pakistan.

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POINT-OF-CARE USE OF LED FLUORESCENCE MICROSCOPY COMBINED WITH ULTRASOUND IN THE DIAGNOSIS OF EXTRA-PULMONARY TB: PRELIMINARY RESULTS

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Extrapulmonary TB (EPTB) is difficult to diagnose and early diagnosis and therapy mean-better prognosis and higher survival rates particularly in patients co-infected with HIV. Ultrasound (US) guided fine needle aspiration is crucial in abdominal EPTB in order to obtain samples for direct microscopy and culture, which remains the reference standard. LED Fluorescence Microscopy (LED-FM) is a potential alternative to conventional fluoroscopy in the identification of Acid Fast Bacilli (AFB). To date, this method has been predominantly used in developing countries, but only on sputum. We tested the use of LED-FM in the diagnosis of EPTB in pus samples collected at the patient's bedside using interventional US. Ten patients who were admitted to the Infectious and Tropical Diseases Unit (San Bortolo Hospital, Vicenza, Italy) with suspected EPTB underwent an US-guided sampling of the lesions. Four of them also had HIV infection. Pus was collected from cervical and axillary lymph nodes in 4 patients, in two patients from hilar hepatic lymph nodes, and from mesenteric lymph nodes, abdominal wall abscess and a psoas muscle abscess in the other cases. The samples were obtained by using a Chiba needle (18 G), and were stained and evaluated using LED-FM. Fluorescing microorganisms were seen in all patients (10/10) with auromine/rhodamine stain. Nine cases were confirmed positive at Acid- Fast examination and at culture. In the negative case, an HIV patient with a history of TB, histoplasmosis was the final diagnosis. LED-FM combined with US shows promise as a rapid point-of-care method for the diagnosis of EPTB, particularly in resource-limited settings where EPTB is highly endemic and laboratories are lacking. Studies on large series are needed to evaluate the sensitivity and specificity of this approach.

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PAPER ANALYTICAL DEVICE TO DETECT SUBSTANDARD ANTI-TUBERCULOSIS MEDICATIONS

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The World Health Organization estimates that up to 10% of the world's pharmaceutical trade consists of counterfeit or substandard drugs; it is thought that up to 25% of the drugs consumed in developing countries are substandard. Substandard medicines are products whose composition and ingredients do not meet the correct scientific specifications. Substandard products may occur as a result of negligence, error, insufficient resources or intentional counterfeiting. Counterfeit antimicrobial drugs are a threat to public health and have been linked to increased mortality and morbidity and emergence of drug resistance. I aim to address this problem by developing a Paper Analytical Device (PAD) to identify substandard medications. PADs are inexpensive, user-friendly, transportable, and require no access to power or technology; thus, ideal for use in a developing country. Each PAD is preloaded with a variety of chemical tests to assess the quality of a medicine; a color change indicates the presence or absence of active pharmaceuticals, excipients and known contaminants. Specifically, I am developing a PAD to detect the quality of the first line of anti-tuberculosis drugs: rifampicin, pyrazinamide, isoniazid, and ethambutol. In the last five years, the USP recorded 20 cases of substandard anti-tuberculosis medications in the Cambodia, Philippines, Vietnam, and Peru. Due to the length of treatment for tuberculosis and existing drug resistance, the standard course of treatment utilizes a combination tablet; therefore, the PAD must not only be able to detect pure forms of the drugs, but also drugs in combination. The battery of colorimetric tests on the PAD indicate the presence or absence of a drug both individually and in combination with the other medications. The PAD will also test for known excipients and other active pharmaceuticals that are known to be used in counterfeiting, such as acetaminophen. My work aims to provide clinics in developing countries with the tools to fight against substandard medications and ensure their health.

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PILOT INTERVENTION STUDY OF HOUSEHOLD VENTILATION AND FINE PARTICULATE MATTER CONCENTRATION IN A LOW-INCOME URBAN AREA, DHAKA, BANGLADESH

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Exposure to fine particulate matter ($PM_{2.5}$) is a risk factor for childhood pneumonia in low income settings. Observational studies indicate that household ventilation may be protective against $PM_{2.5}$ exposure. In this pilot study, we tested the effects of behavioral and structural interventions to increase household ventilation on indoor $PM_{2.5}$ in Kamalapur, an urban slum in Dhaka, Bangladesh with infrequent biofuel use. We conducted the pilot between July and August 2011, the hot season when windows and doors are commonly open and fans are on. We recruited good ventilation (window or door in ≥3 walls) and poor ventilation (no window, 1 door) homes using stratified random sampling. We monitored $PM_{2.5}$ for 48 hours inside and outside each home at baseline. We asked participants to increase ventilation behavior by opening windows and doors and turning fans on as much as possible and to keep logs of these behaviors. When physically feasible and permitted, we installed a window in poor ventilation homes. $PM_{2.5}$ monitoring was repeated after behavioral intervention and window installation. We used linear mixed effects models to examine the effect of behavioral and structural interventions

on $PM_{2.5}$ concentrations. We estimated the number of hours that $PM_{2.5}$ concentrations exceeded 50, 100, 250, 500, and 1000 μg/m^3. Compared to 29 poor ventilation homes, 59 good ventilation homes were larger and more likely to be made of concrete; their residents were wealthier, better educated, and more likely to never use biofuel. Among poor ventilation homes, installation of a window was refused by the landlord (n=6, 32%) or resident (n=5, 26%), or we were unable to install the window due to space constraints (n=4, 21%). A window (mean size 0.4m^2) was installed in 10 (34%) homes. After adjusting for covariates, the addition of a new window in poor ventilation households was inversely associated with the number of hours in the 48-hour monitoring period that indoor $PM_{2.5}$ exceeded 250, 500, and 1000μg/m^3 (3.1, 3.9, 3.2 hrs, respectively, $p<0.05$). Compared to baseline there was no significant difference after intervention in the mean number of hours that each door (17.3 v 18.2) and window (18.5 v 19.4 hrs) was open per day. Installation of a window was associated with reduced indoor $PM_{2.5}$, but recommendations to increase ventilation behavior were not. When feasible and acceptable, installation of a small window may help to improve air quality in low income homes.

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PANDEMIC H1N1 INFLUENZA SURVEILLANCE IN HAITI, JULY-DECEMBER 2009

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Influenza is a significant cause of morbidity worldwide, and little is known about influenza in the Caribbean region. In June 2009, following the WHO declaration of influenza A (H1N1) pdm09 (2009 H1N1) pandemic, Haiti established a sentinel surveillance system for influenza. Providers were instructed to collect nasopharyngeal specimens from patients with influenza-like illness (defined as a person with a temperature >38C and cough or sore throat in the absence of other diagnoses) at health facilities in all 10 departments of Haiti. Beginning in July 2009, the Haiti National Public Health Laboratory tested specimens for influenza A and B using real-time RT-PCR and subtyped for pandemic influenza, A/H3 and seasonal A/H1. A subset of samples was sent to CDC-Atlanta for confirmatory PCR and antiviral resistance testing using pyrosequencing and neuraminidase inhibition assays. During July-December 2009, 509 specimens from all 10 departments were collected. 10 were discarded because of lack of identifying information. The majority were from the Ouest department (73%). 197/499 (39.5%) specimens were positive for influenza: 95 (48%) were pandemic 2009 H1N1, 57 (29%) were seasonal influenza A/H3N2 and 45 (23%) were influenza B. The median age of 2009 H1N1 influenza patients was 21.7 years (range: 5 months- 67 years). Two-thirds of 2009 H1N1 were in patients aged 6 months - 35 years. There was no significant difference in the mean ages of patients with 2009 H1N1 and seasonal influenza (22 vs. 25.5, p=0.1). Pandemic 2009 H1N1 activity in Haiti peaked in September. All 11 2009 H1N1, four seasonal and two influenza B specimens tested were susceptible to neuraminidase inhibitors. In Haiti, as in other countries in the region, 2009 H1N1 influenza affected mostly children and younger adults and peaked in early Fall 2009. Additionally, Haiti had co-circulation of influenza subtypes and types.

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EPIDEMIOLOGY OF PNEUMOCOCCAL PNEUMONIA IN ADULTS IN GUATEMALA

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Streptococcus pneumoniae is an important cause of pneumonia worldwide, but little is known about the epidemiology of pneumococcal disease in adults in Latin America due to diagnostic challenges. To describe the epidemiology of pneumococcal pneumonia in adults, we analyzed surveillance data from Santa Rosa and Quetzaltenango in Guatemala during 2008-2011. A case of pneumococcal pneumonia was defined as a hospitalized patient aged ≥18 years with evidence of acute infection (fever, hypothermia, or abnormal leukocyte count or differential), at least one sign or symptom of respiratory disease, and either a rapid urinary antigen test or a blood culture positive for *S. pneumoniae*. We calculated incidence rates for municipalities near the hospitals based on growth-adjusted population denominators from census data. The surveillance system captured 938 adults with evidence of acute infection and a sign/symptom of respiratory disease; 544 (58%) underwent urinary antigen testing and 176 (19%) had blood culture performed. A total of 101 cases of pneumococcal pneumonia were detected; all had positive urinary antigen test and four additionally had *S. pneumoniae* isolated from blood culture. Chest radiograph results were available for 63 pneumococcal pneumonia cases, of which 42 (66%) showed radiologic evidence of pneumonia. The incidence rate of pneumococcal pneumonia was 6.4 per 100 000 persons per year: 3.7 among persons 18-39 years; 5.2 among persons 40-64 years; and 24.7 among persons ≥ 65 years. The case fatality proportion was 8%: 11% (4) among persons 18-39 years; 4% (1) among persons 40-64 years; and 8% (3) among persons ≥ 65 years. Observed rates likely underestimate burden of pneumococcal disease but highlight increased risk among elderly people. These data provide a useful baseline against which to measure the indirect impact of introducing the pneumococcal conjugate vaccine in infants, which has lead to declines in adult pneumococcal disease in other settings.

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HEALTHCARE ASSOCIATED RESPIRATORY SYNCYTIAL VIRUS INFECTIONS AT THREE REFERRAL HOSPITALS IN KENYA, SEPTEMBER 2009 - JULY 2011

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Respiratory Syncytial Virus (RSV) is a major cause of respiratory illness in all ages but occur more often in children < 1 year. RSV presents as a mild infection but can cause severe illness. Children < 2 years with chronic heart or lung disease and Immunocompromized individuals are at high risk of severe RSV infection. RSV is the most common cause of respiratory hospital acquired infection (rHAI) in pediatric wards with up to 45.0% of contacts getting infected. There is limited information on the role of RSV in rHAI in developing countries. This study was conducted to determine the genetic relationship of RSV strains in order to confirm nosocomial transmission from three Kenyan referral hospitals [Kenyatta National

Hospital (KNH), New Nyanza General Hospital (NNPGH) and Mbagathi District Hospital (MDH)]. Nasopharyngeal and oropharyngeal samples were collected from hospitalized patients who developed respiratory illness, 72 hours post admission. A total of 286 samples were screened for the presence of RSV matrix protein using real-time RT-PCR and characterized as RSV-A and -B using a multiplex real-time RT-PCR assay. Of these, 252 had complete demographic data and were included in further analysis. Sixty percent of the patients were from KNH, 22.5% were from NNPGH, and 17.5% were from MDH. RSV was detected in 40 (15.9%) patients with 22 typing as either A (59%), B (31.8%) or AB dual (9.1%) subtype. Seventeen of these positive samples were successfully sequenced. Three RSV- A and 2 RSV-B sequenced samples from KNH were 100% identical in the G ectodomain sequences. One RSV-A specimen from MDH and one RVS-A positive from NNPGH had 100% identity although these two hospitals are approximately 160 miles apart. Three sequences from KNH clustered together with high nucleotide sequence identity as well as high bootstrap support suggesting a common source virus. These results suggest that there were possible multiple introductions of RSV into these hospitals as well as some possible spread of a single strain within one hospital. It is therefore recommended that there should be enhanced infection control measures in hospitals to curb the spread of RSV and other infections in the study areas.

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ETIOLOGY AND IMPACT OF RESPIRATORY INFECTIONS DURING PREGNANCY AND INFANCY ON THE HEALTH OF MOTHERS, FETUSES AND NEWBORNS: A PROSPECTIVE COMMUNITY-BASED SURVEILLANCE STUDY IN PAKISTAN

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In the developing countries, the etiology of common respiratory infections during pregnancy, and the impact of these infections on the health of the mother and fetus are not known. Similarly, the etiology of respiratory illness and fever in newborns, and their impact on the growth and development during the infancy needs to be better understood. This knowledge is important to make appropriate preventive strategies like vaccination during pregnancy and infancy. Our study aims to determine the etiologies of ARI and febrile illness in pregnant women and their newborns in Pakistan, and to determine the long term impact of these infections on the health of mothers, and the growth and development of fetuses and newborns. We are conducting a three-year longitudinal cohort observational study in Bilal Colony, an urban slum of Karachi, Pakistan, where we are following a cohort of 350 pregnant women from the first trimester onwards, and their newborns from birth through the duration of the study period. The study participants are visited every week to check for any ARI episodes or fever. Blood cultures and malaria ICT are obtained for fever > 100.4˚F/38˚C, and nasopharyngeal swabs are obtained for respiratory viral detection in eligible ARI episodes. Anthropometric measures are obtained from all study participants on monthly basis. In the first 6 months of this study so far, the enrollment of pregnant women has been completed, and 123 women have delivered. Out of these, there have been 59 live births, 6 still births and 30 spontaneous abortions. The spontaneous abortions rate is higher than the reported national estimate of abortions, which is about 1 in 7 pregnancies. So far, there have been 3 fever and 3 ARI episodes in pregnant women, and one case of severe pneumonia in the newborn. Continued surveillance of this cohort will enable us to better define the etiologies of ARI and fever in pregnant women and newborns in Pakistan, and the long term impact of these infections on the health of mother, fetus and newborn.

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BIRTH COHORT TO STUDY INFLUENZA INFECTIONS IN INFANTS 0 TO 2 YEARS OLD IN NICARAGUA

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Influenza is a major public health problem worldwide; however, relatively few data exist on influenza in tropical regions, especially in very young children. In September 2011, the Nicaragua Influenza Birth Cohort Study was established in Managua, Nicaragua, to study the burden of influenza in infants and toddlers. The study design allows for the investigation of repeat infections, including immunological characterization, as well as determinants of disease severity. We plan to conduct the study for three years, with 250 infants enrolled each year and followed until 2 years of age, for a total sample size of 750 infants. Each month, approximately 20 newborns, aged 4 weeks or less, are enrolled in the study to maintain the age structure. Infants are contacted on a weekly basis by study personnel, and data on daily symptoms are collected using diary cards. Children are provided with all primary medical care through the study, and 189 variables are collected at each medical visit. All participants presenting with fever or reported fever are tested for influenza by RT-PCR. Yearly blood samples are collected beginning at 6 months of age to enable detection of influenza infections and to evaluate nutritional status. Numerous information and communications technologies are employed to manage study data, track samples, and maintain quality control, including smart phones, tablets, barcodes, global information system (GIS), and scanable forms. We are currently piloting the direct entry of medical visits into tablet computers. To date, acceptance into the study has been high (89.0%), and loss to follow-up/withdrawal has been low (3.9%). As of April 2012, 154 infants have been enrolled, with a median age at enrollment of 17 days. The participants attended 855 medical visits at the study health center. Although the study period to date has not included the influenza season, two laboratory-confirmed influenza cases were detected. A total of 35 participants were transferred to the hospital. Twenty percent of the transfers to the hospital were for respiratory illness; one infant was transferred for bronchitis and 7 for pneumonia. One infant died of pneumonia. In September of 2012, we will complete the first year of the study and perform an interim analysis of results. This study will provide crucial information about the burden of influenza and the incidence and characteristics of sequential influenza infections in infants.

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BIBLIOMETRIC ANALYSIS OF THE IMPACT OF THE 2009 INFLUENZA PANDEMIC ON THE SCIENTIFIC LITERATURE PRODUCTION ON INFLUENZA A

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Influenza A virus causes recurrent pandemics. Concern over avian influenza (H5N1) in recent decades has heightened attention on influenza, spurring greater investment in research and public health preparedness. When the influenza A H1N1 virus (pH1N1) pandemic occurred in 2009, the response was rapid and a large amount of scientific information was published. Bibliometric analysis is a systematic method to evaluate research output through quantity and quality methods. We conducted a bibliometric analysis to measure the impact of the 2009 pH1N1 pandemic on scientific literature production as measured by the immediacy index (II), a measure of how quickly articles in a journal are cited, and impact factor (IF), a measure reflecting the average number of recent citations. A search strategy was built to retrieve original studies on influenza published from

2005-2010 in the PubMed database. II and IF of journals were obtained from the Journal Citation Report. During the study period, 7,046 original studies related to influenza were published in 1,009 journals from 85 countries. 1,980 studies (28%) obtained funding and 145 (2%) were registered as group authorship. The number of original studies, involved journals, funded studies, and group authorships trended upward during the study period, with accentuated growth from 2009. Studies published in 2009 appeared in journals with higher II and IF than previous years, returning to pre-pandemic levels in 2010. Journals that published a high percentage of studies on influenza had greater increases in II (p=0.02) in 2009 and more than twice the number of citations compared to 2008. Increased IF was observed in 2009 for all journals independent of the percentage of influenza studies published (p=0.88). Scientific literature production regarding influenza was increasing even before the 2009 pH1N1 pandemic, partly as a result of concern over avian influenza. However, the pH1N1 pandemic caused a significant increase in influenza research as reflected by increases in the number of studies, participation of journals, funding, and creation of research groups.

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HOUSEHOLD AIR QUALITY IN RURAL PERU: EFFECTIVENESS OF AN IMPROVED COOKING STOVE TO REDUCE EXPOSURE TO INCOMPLETE BIOMASS COMBUSTION

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Half of the world's population depends on biomass fuels to meet domestic energy needs. High levels of emitted pollutants are responsible for substantial morbidity and mortality from lower respiratory infections globally, particularly from household air pollution. The study was embedded in a community-randomised controlled trial carried out in the northern highland, Peru. We determined 48hr household air concentration levels of particulate matter (PM2.5), carbon monoxide (CO) in 93 kitchen environments and personal exposure, seven months after an improved stove (OPTIMA) was installed. We measured simultaneously the personal exposure of mothers and household air in the kitchen using Draeger Pac III datalogger for real-time CO and particle-size-selective Triplex Cyclones for 48hr time-integrated PM2.5 concentrations. Housing characteristics and stove functionality were assessed through questionnaires and participatory observations. Indoor kitchen concentration of PM2.5 and CO did not differ significantly between improved-chimney stoves and traditional stoves. However a post-hoc stratification by functionality levels revealed mean PM2.5 and CO emissions of the well-maintained improved stoves were 28% lower (n=20, PM2.5, 135µg/m3 95%CI 54-216) and 45% lower (n=25, CO, 3.2ppm, 95%CI 1.5-4.9) in the kitchen environment compared to the control stoves (n=34, PM2.5, 188µg/m3, 95%CI 115-261; n=44, CO, 5.8ppm, 95%CI 3.3-8.2), though no statistical significance was observed. Likewise, personal exposures were 44% and 17% lower for PM2.5 (n=23) and CO (n=25). Functionality levels, kitchen volume, type of wood used and the duration the stove was lit were significantly associated with PM2.5 and CO levels in the kitchen, while functionality levels, hours the stove remained lit and the mothers perceiving smoke as a nuisance and contaminant of the kitchen environment were predictors for PM2.5 and CO personal exposure. At the time of sampling, 66% (28/43) of the improved stoves were properly maintained. The results underscore the importance of not only measuring success of household air pollution programmes by the number of installed chimney stoves, but rather by assessing quality of the installation and maintenance of the devices, adoption and continuous use over time. Improved stove programmes should consider both, determinants for sustainability- and functionality in designing future sustainable interventions.

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INFLUENZA-ASSOCIATED SEVERE PNEUMONIA RATES IN CHILDREN YOUNGER THAN FIVE YEARS IN EL SALVADOR DURING 2008-2010 SEASONS

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Disease burden attributable to influenza is poorly understood in tropical low-income countries. To guide health policy, we estimated rates of severe pneumonia associated with influenza among children aged < 5 years in El Salvador. Each week, physicians identified and collected data on all children aged <5 years-old hospitalized with severe pneumonia. Nasal and oropharyngeal swabs were collected for influenza testing on a convenience sample of case-patients. We conducted a health utilization survey in the hospital catchment area to determine the proportion of residents who sought care at the sentinel-site. We estimated rates of severe pneumonia using surveillance and census data adjusting for health service utilization. Influenza strains were characterized by CDC. Influenza strains were compared with northern hemisphere and southern hemisphere vaccine formulations. Physicians identified 2,554 severe pneumonia of which 6% (37/608) of those tested were positive for influenza. The rate of severe pneumonia among children <5 years-old was 1.5/1,000 person-years (py) during 2008, 7.6/1,000 py during 2009 and 0.6/1,000 py, during 2010. Both northern and southern hemisphere vaccine formulations matched isolated influenza virus strains during 2008 and 2010. Influenza hospitalizations were common in El Salvador, but incidence varied substantially among influenza seasons. Both northern and southern hemisphere influenza vaccines were well-matched to circulating strains. Consideration of expansion of influenza vaccination programs may be warranted.

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THE SOCIAL AND ECONOMIC COST OF UNDERGOING TREATMENT AS A TB PATIENT IN GHANA

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Although TB treatment in Ghana is free anecdotal reports indicate that economic and social costs affect their ability to seek appropriate health care. TB programs therefore need to ensure that the economically and socially disadvantaged groups do not face barriers that keep them from seeking treatment. The Poverty Sub-Working Group of the Stop TB Partnership developed a tool which can assist TB programs estimate the costs of TB patients before and during diagnosis and during treatment. This tool was implemented by the Dodowa Health Research Centre and the National TB control Program in Ghana to estimate the costs of TB patients before and during diagnosis and during treatment. It was implemented in all districts in the Upper East region (a deprived region) and two districts in the Eastern region (a better endowed region) and

was administered to new TB patients, older than 15 years, who had received at least one month of TB treatment and given consent. Data was collected through informal discussions with TB coordinators and facility heads, desk top review of patient records and intervies with TB patients to assess direct costs prior to being diagnosed and direct and indirect cost of current treatment. Of the 159 patients interviewed, 64% were in the three lower socio economic quintiles with monthly income less than US$ 42.55. Health system delay was estimated at 1.4 weeks with males taking longer to seek care than females. DOTS patients paid a mean total direct cost of US$ 0.50 for each visit and spent of 58 minutes per DOTS visit. The mean number of days spent in the hospital was 22.7 days and direct cost of hospitalization was US$ 48.32. Whilst 48.3% of the patients borrowed, 37.7% sold assets to cope with paying for their ill health. Reduction of monthly household and patients income due to TB was 44.5% and 82.6% respectively. Sixty-one percent of the TB patients lost their jobs, 11% got separated from their spouse/family and stopped attending public functions. Through this study, the NTP has identified constraints faced by TB patients and their families that have an effect on case finding and treatment adherence. We recommend that TB patients and their families should benefit from social protection packages that will ease the financial burden. Employers should not hesitate to take back workers who have been diagnosed and treated with TB.

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CLINICAL OUTCOMES ASSOCIATED WITH ROUTINE USE OF INTERFERON-Γ RELEASE ASSAYS IN A CENTRAL LONDON TB SERVICE

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Interferon-γ release assay (IGRA) has been shown to have higher specificity than tuberculin skin testing (TST) for screening for latent TB and is recommended by the National Institute for Clinical Excellence (NICE). The aim of this study is to review the indications for and analyse the results of IGRA testing in our central London BCG vaccine positive population. We analysed routinely collected clinical and demographic data on patients referred for IGRA testing to a TB service at a large London teaching hospital from September 2007 to January 2012. Reasons for referral for screening included contacts of active TB, pre-monoclonal antibody therapy, recent migrants and occupational health. Quantiferon Gold InTube was used. We used the London TB registrar to identify patients that were diagnosed with active TB either by bacteriological or clinical evidence from November 2007 to February 2012. We determined the sensitivity and specificity of IGRA for diagnosing active TB and screening for latent TB. We used univariate linear regression to assess the incremental impact of IGRA result on having a diagnosis of active TB. 961 IGRAs were performed on 917 patients. 51% were male with a median age of 30 years (IQR 19-40). There were 46 (4.8%) indeterminate, 703 (73.2%) negative and 212 (22%) positive results. Indeterminate results were more prevalent amongst immunosuppressed than immunocompetent patients 66.7% (24/36) vs. 33.3% (12/36). 46 cases of active tuberculosis were identified from the London tuberculosis register, 15 of which had a negative IGRA result. The sensitivity and specificity of IGRA for diagnosing active TB were 67% and 79% respectively. We found a direct correlation between a positive IGRA test result and active TB diagnosis (p 0.00 coefficient 1.343). The sensitivity and specificity of IGRA for latent TB screening were 55% and 89% respectively. We found an overrepresentation of indeterminate results amongst immunosuppressed patients. IGRA was used in addition or other conventional diagnostic modalities for the diagnosis of active TB, which is outside the scope of the NICE guidelines. Although the use of IGRA for this purpose is approved by the US Food and Drug Administration, caution should be exercised due to its low sensitivity for diagnosing active TB. However the usefulness of IGRA in screening for latent TB was conferred by its high specificity in our setting.

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EVALUATION OF HOUSEHOLD LEVEL INTERVENTIONS DURING A LARGE, URBAN TYPHOID FEVER OUTBREAK - HARARE, ZIMBABWE 2011-2012

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Between October 2011 and March 2012, ~2,750 suspected cases of typhoid fever in two high-density suburbs of Harare (Dzivaresekwa and Kuwadzana) were reported to the Harare City Health Department (HCHD). To prevent outbreak spread, HCHD and non-governmental organizations conducted door-to-door health and hygiene education and distributed point-of-use water treatment (PoUWT) products beginning in October 2011. To evaluate the effectiveness of these interventions, we conducted cross-sectional household surveys in these two affected suburbs in March 2012, including free chlorine residual (FCR) testing in stored drinking water. Reported intervention coverage was high, with 351 (77%) of 458 randomly selected households having received both typhoid fever prevention information and at least one PoUWT product. Of 368 households that received at least one of the three types of chlorine tablets distributed, 326 (89%) reported ever using them, 160 (43%) reported using them daily, and 98 (27%) had stored water that was treated and had FCR ≥0.2mg/L when tested. Only 169 (55%) of 310 household respondents who had chlorine tablets on the day of the survey knew the correct volume of water to treat with their tablets. In univariate analysis, respondents who had higher income, were older, had received PoUWT products or typhoid fever prevention information, and who reported household water treatment before the outbreak were more likely, and respondents who reported boreholes as the primary source of drinking water were less likely, to report water treatment during the outbreak or on the day of the survey, and to have treated stored water with FCR ≥0.2mg/L ($p < 0.05$). The findings highlight: 1) relatively low uptake of PoUWT after free distribution (consistent with other research); 2) the need to improve coordination of NGO response activities through consistent PoUWT product choices and communication about product use; and, 3) the need to emphasize treating drinking water from all sources daily to control and prevent typhoid fever and other waterborne disease outbreaks.

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EFFECTS OF ENVIRONMENT ON HUMAN CYTOKINE RESPONSES: ROLE OF URBAN VERSUS RURAL RESIDENCE

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Environment may have a key role in the development of the immune system in childhood and may explain the low prevalence of allergic and autoimmune diseases in the rural tropics. To investigate the effects of urban versus rural residence on the immune response, we recruited 440 school children living in either in rural communities in the Province of Esmeraldas or in the city of Esmeraldas. We collected data on